ABSTRACT

SERUM PROLACTIN AND GROWTH HORMONE CONCENTRATIONS FOLLOWING THYROTROPIN RELEASING HORMONE IN CATTLE DURING VARIOUS PHYSIOLOGICAL STATES

By

Dwight T. Vines

Serum prolactin and growth hormone (GH) concentrations were determined following administration of thyrotropin releasing hormone (TRH) during various physiological states.

In eight prepubertal heifers prolactin concentrations before TRH averaged 16 ng/ml and increased to a maximum value of 81 ng/ml following TRH administration. Repeatability coefficients for day to day baseline prolactin concentrations and prolactin response curve areas (integrated area of plot of hormone concentrations for 30 min after TRH minus baselines before TRH) following TRH administration were .27 and .61, respectively. These results suggest that there are great differences from day to day in baseline prolactin concentrations and that there is less day to day variation in prolactin response curve areas following TRH administration. However, the correlation coefficient, within days, between baseline and maximum serum prolactin concentrations following TRH administration was .64 ($P<.01$).

Serum GH concentrations in prepubertal heifers prior to TRH averaged 8 ng/ml and increased to a maximum of 21 ng/ml after TRH.

Based on averaged GH response curve areas, there was little day to day variation in pituitary responsiveness to TRH. However, because of animal variation, averaged GH response curve areas are misleading. This is demonstrated by the low repeatability coefficient (.35) for GH response curve areas following TRH. Similarly, the day to day repeatability coefficient for baseline GH concentration was low (.37). Correlation coefficient between baseline GH and post-TRH maximum GH concentrations was .60 ($P<.01$). Similar to prolactin, maximum GH concentrations after TRH on a given day are associated with baseline GH concentrations.

Routes of administration (IV, intramuscular (IM) and subcutaneous (SC)) of TRH affected ($P<.01$) the quantities (response curve areas) of prolactin and GH released during a 30-minute post-TRH sampling period in six prepubertal heifers. In contrast, during a 2-hr post-TRH sampling period, neither prolactin nor GH response curve areas were different ($P>.05$) following IV or SC administration of TRH. Following IV administration of TRH, serum prolactin and GH concentrations reached peak values more rapidly but declined more precipitously than following IM or SC administration of TRH. Therefore, when a prolonged hormonal response is desired, the SC or IM route of administration of TRH should be used.

Dose of TRH affected prolactin and GH response curve areas in six prepubertal heifers. The quantities (post-TRH response curve areas) of prolactin (1515 to 3940 ng $ml^{-1}min$) and GH (53 to 667 ng $ml^{-1}min$) increased linearly ($P<.01$) with increases in the log of the dose (5 to 100 μg) of TRH. Peak serum prolactin concentrations increased from an averaged pre-TRH concentration of 39 ng/ml to values of 143 to 277 ng/ml

after TRH. Maximum quantities of prolactin released were achieved with 50 µg TRH. Pre-TRH GH concentrations averaged 5 ng/ml and increased to values of 11 to 46 ng/ml following TRH administration. Unlike prolactin, the largest GH response curve areas were attained using 100 µg TRH, and it is possible that a larger dose of TRH would result in still greater release of GH.

Serum prolactin and GH response curve areas did not differ ($P>.05$) following administration of TRH on various days of the estrous cycle in six heifers. Peak serum prolactin and GH concentrations increased from averaged pre-TRH concentrations of 6 and 6 ng/ml to 70 and 21 ng/ml after TRH, respectively. Prolactin response curve areas, pre-TRH concentrations and maximum post-TRH concentrations at days 2 and 4 of the estrous cycle "tended" to be less than those at other stages of the estrous cycle.

During pregnancy, prolactin response curve areas were not affected ($P>.05$) by stage of pregnancy following TRH administration in 26 heifers. However, there was a "tendency" for increased pre-TRH prolactin concentrations, maximum post-TRH prolactin concentrations and prolactin response curve areas with advancing pregnancy. Pre-TRH prolactin and GH concentrations averaged 14 and 4 ng/ml and increased to values of 152 and 11 ng/ml following TRH administration, respectively. Prolactin response curve areas were affected ($P<.01$) by season of year, being 6 to 15 X greater in summer than in fall or spring.

Prolactin response curve areas were not affected ($P>.05$) by stage of lactation (2, 4, 6, 8 and 10 months) or month of pregnancy in 16 lactating cows following TRH administration. However, there "tended" to be greater prolactin release at the 2-month stage of lactation. Peak

serum prolactin and GH concentrations increased from averaged pre-TRH concentrations of 16 and 5 ng/ml to values of 234 and 16 ng/ml after TRH, respectively. Month of year affected ($P<.01$) prolactin response curve areas following TRH administration. GH response curve areas were greater ($P<.05$) at the 2-month stage of lactation when compared with other stages. These data suggest that the capacity of the pituitary to release prolactin and GH after administration of TRH is greater during early lactation and that this increased responsiveness may be associated with increased milk yields during early lactation. However, overall correlations between milk production (average of 5 days preceding TRH injections) and hormone response curve areas at the five stages of lactation were -.01 and .15 ($P>.05$) for prolactin and GH, respectively.

Neither month of pregnancy nor month of year affected ($P>.05$) GH response curve areas following TRH administration to pregnant heifers or lactating cows.

In overview, season of the year is more important in determining serum prolactin, but not GH, concentrations than are any of the different physiological states studied. Furthermore, physiological states which I examined have relatively little effect on basal or TRH-induced releases of serum prolactin or GH.

SERUM PROLACTIN AND GROWTH HORMONE CONCENTRATIONS FOLLOWING THYROTROPIN RELEASING HORMONE IN CATTLE DURING VARIOUS PHYSIOLOGICAL STATES

By

Dwight T. Vines

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Dairy Science

1976

PERSONAL NOTE

As man ventures forth, let not his brother be overly critical; yet, let man heed his brother's advice and suggestions. For man is not an island, but a part of the body of mankind who must contribute to mankind's knowledge of life or life will surely die. Let man also honor his father and mother for without them he would not be.

I pay tribute to my parents' many sacrifices and their encouragement and hereby dedicate this thesis to them.

ACKNOWLEDGMENTS

The author wishes to express his appreciation to the Department of Dairy Science for providing funds and facilities for his graduate studies. A debt of gratitude is extended to Drs. J. H. Britt, H. D. Hafs, R. Mellenberger and W. D. Oxender for their advice throughout my graduate program. He also would like to express his appreciation for the advice and support of his committee members, Drs. L. Bieber, J. Gill, G. Riegle and ex officio member, E. M. Convey. He expresses a particular debt of gratitude to his major professor, Dr. H. A. Tucker, for his advice, understanding and perseverance in times of difficulty and indecision and for his assistance in preparing this thesis.

The author wishes to express a great debt of gratitude to Dr. R. Neitzel for his computer programming assistance and to his fellow graduate students for all-around assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Milk and dairy products are excellent sources of protein, fats, carbohydrates, minerals and vitamins. With an ever increasing world population that has already surpassed four billion, sources of high quality foodstuffs are of prime importance. But presently many of the world's population consume little or no dairy foodstuffs, due to their high cost or unavailability. One of the factors necessary to make more dairy foodstuffs available is more efficient production of these foodstuffs.

In the recent past, the approach to increasing production or the efficiency of production was through either more efficient feeding and management or improvement of the milking potential through genetic selection. Both of these approaches have been worthwhile, since milk production per cow continues to increase each year.

However, only very recently has the endocrinological approach to increasing production come to the forefront. Today, we know that growth, reproduction, and lactation are under control of the endocrine system. Prolactin and growth hormone in laboratory species have been implicated as part of a complex of hormones which are essential for mammary growth, lactogenesis and maintenance of lactation. However, their role in lactation in the bovine is still unclear. Therefore, the

purpose of these studies was to determine the pituitary's responsiveness, as measured by serum concentrations of prolactin and growth hormone, to exogenous administration of a naturally occurring hypothalamic releasing factor during various physiological conditions. This information should give us insight into the control of prolactin and growth hormone secretion and hopefully produce information that can be applied subsequently to increase milk production.

REVIEW OF LITERATURE

Prolactin and Growth Hormone Requirements for Lactation

Pioneering experiments by Stricker and Grueter (1928) demonstrated that injection of aqueous extracts of anterior pituitaries induced milk secretion in pseudopregnant rabbits. This report suggested that some hormones of the anterior pituitary may be important in the initation of lactation.

Following the identification and isolation of prolactin (Riddle et al., 1933) and growth hormone (Li and Evans, 1944), many investigations were begun to ascertain the function of these two hormones with regard to lactation. Daily injections of sheep prolactin into hypophysectomized rabbits induced milk secretion (Fredrikson, 1939). Lyons (1942) confirmed these results when he demonstrated that a purified ovine anterior pituitary preparation, capable of inducing localized proliferation of the pigeon crop-sac mucosa, initiated milk secretion following injection into a single galactophore of the rabbit mammary gland. Kilpatrick et al. (1964) also induced lactation with ovine prolactin injection in hypophysectomized, pseudopregnant rabbits. However, prolactin injections alone are not capable of inducing lactation in rats (Talwalker et al., 1961) or mice (Nandi and Bern, 1961). Cowie (1969) reviewed his work and that of others and concluded that either prolactin or GH in combination with adrenocorticotropic hormone

(ACTH) or adrenal corticosteroids would induce lactation in hypophysectomized, adrenalectomized and ovariectomized rats and mice.

Prolactin has not been shown to be galactopoietic in dairy cows or ewes (Folley and Young, 1940, Sulman and Twersky, 1948, Wrenn and Sykes, 1953, Delouis and Denamur, 1967 and Morag et al., 1971). However, Gotsulenko (1968) observed increased lactational performance in goats following injection of prolactin into the arterial system of the mammary gland.

Following injection of ergot alkaloids, which block prolactin release from the pituitary, lactation was depressed in rabbits (Taylor and Peaker, 1976) and rats (Shaar and Clemens, 1972 and Tomogane et al., 1975). However, milk yields were not affected by ergot drugs during established lactation in goats (Hart, 1973) or cows (Karg et al., 1972 and Smith et al., 1974) although serum prolactin concentrations were markedly depressed. Koprowski and Tucker (1973) reported a significant correlation between the magnitude of prolactin released to the milking stimulus and milk production in cows which suggests that prolactin released at milking may influence subsequent milk yields as has been reported for rats (Grosvenor and Mena, 1973).

Cowie (1969) reported that prolactin, GH, corticoids and triiodothyronine were necessary for complete restoration of lactation in hypophysectomized goats, although prolactin alone was slightly lactogenic. However, removal of prolactin following restoration of lactation had no deleterious effects on the subsequent lactation, but removal of GH resulted in an immediate decline in milk yield. Furthermore, administration of GH enhanced lactational performance in

cows (Cotes et al., 1949, Donker and Peterson, 1951, Chung et al., 1953, Wrenn and Sykes, 1953, Bullis et al., 1965 and Machlin, 1973).

Prolactin and Growth Hormone Response to Milking, Suckling and Teat Stimulation

Milking, suckling and teat or udder manipulation have been associated with increased serum prolactin concentrations. Bryant et al. (1968) reported a three-fold or more increase in serum prolactin following milking in goats. Johke (1970) reported a 40- to 100-fold increase in serum prolactin associated with the milking stimulus in goats. The suckling stimulus released prolactin in ewes (Lamming et al., 1972 and McNeilly et al., 1972) and cows (Fell et al., 1971). Teat stimulation or udder manipulation resulted in prolactin release in cows (Johke, 1970, Fell et al., 1971, Tucker, 1971, Schams, 1972 and Reinhardt and Schams, 1975) and goats (Johke, 1970). Reinhardt and Schams (1975) reported a 3- to 10-fold increase in serum prolactin in four heifers following a 3 minute stimulation of two teats.

Johke (1970) reported a rapid increase in serum prolactin associated with the milking stimulus in cows. Peak concentrations were reached 4 to 20 minutes after the start of milking. Prolactin release in response to the milking stimulus decreased with advancing stages of lactation. Tucker (1971) also reported a rapid increase in serum prolactin associated with the milking stimulus. Like Johke (1970), Koprowski and Tucker (1973) found a diminished prolactin response to milking in late lactation.

Thyrotropin releasing hormone (TRH) releases prolactin in lactating cows (Convey et al., 1973 and Kelly et al., 1973). Tucker et al. (1975) reported a rapid increase in serum prolactin with peak

concentrations attained 30 minutes after the beginning of a constant infusion of TRH in lactating cows. However, serum prolactin concentrations declined from peak values during 6 to 13 hours of continuous TRH infusion. In addition, Reinhardt and Schams (1975) reported eventual declines in serum prolactin concentrations from peak concentrations under continuous TRH infusion in heifers. However, when the milking stimulus (Tucker et al., 1975) or teat stimulation (Reinhardt and Schams, 1975) was superimposed on declining serum prolactin concentrations during continuous TRH infusion, markedly increased serum prolactin concentrations were re-established. One possible explanation for these data is that milking or teat stimulation probably act through the hypothalamus to release prolactin while TRH probably acts directly on the pituitary to release prolactin. Therefore, the diminished prolactin response to continuous infusion of TRH may be due to refractoriness of the pituitary to TRH while the prolactin response to milking or teat stimulation is unaffected.

Tucker (1971) reported lack of a GH surge after application of the milking stimulus in cows. Koprowski and Tucker (1973) confirmed these results when they were unable to detect a GH surge after application of the milking stimulus for the duration of lactation in 26 cows. However, Hart and Flux (1973) reported increased serum GH in goats in response to the milking stimulus. This difference in response may be due to species variability.

Prolactin and Growth Hormone Response Following TRH

Isolation of porcine TRH (Schally et al., 1969) was followed quickly with elucidation of its structure (Nair et al., 1970) and with synthesis in the laboratory (Boler et al., 1969).

Tashjian et al. (1971) first reported release of prolactin from cloned rat pituitary tumor cells in culture following TRH. These data suggested to me that TRH might be used as an exogenous and physiological stimulus, since TRH is a naturally occurring hypothalamic releasing factor, to evaluate the capacity of the anterior pituitary to release prolactin.

Jacobs et al. (1971) reported an 11-fold increase in serum prolactin over fasting levels in humans following TRH. Maximal values were reached 15 to 20 minutes post-TRH. Bowers et al. (1971) also reported increased serum prolactin following TRH in humans. These early reports were confirmed by several researchers (Friesen et al., 1972, L'Hermite et al., 1972, Bowers et al., 1972, Gautvik et al., 1973, Hershman et al., 1973 and Noel et al., 1974).

Administration of TRH to rats increased serum prolactin (Deis and Alonso, 1973, Mueller et al., 1973, Blake, 1974 and Porteus and Malven, 1974). However, Lu et al. (1972) reported no increase in serum prolactin following administration of 50 μg TRH for six days although there was a significant increase in pituitary prolactin content. Although these results are contradictory, the increase in pituitary prolactin content may indicate that TRH enhances prolactin synthesis. Following administration of TRH, serum prolactin increased in sheep (Davis and Borger, 1972, Debeljuk et al., 1973, Fell et al., 1973 and

Moseley et al., 1973) and in cattle (Schams, 1972a, Convey et al., 1973 and Kelley et al., 1973).

Takahara et al. (1974) observed increased serum GH in rats following infusion of TRH into a hypophysial portal vessel. Udeschini et al. (1976) also reported increased serum GH following TRH in hypophysectomized rats bearing an ectopic pituitary. Convey et al. (1973) observed that GH increased proportionally to the dose of TRH in lactating cows, whereas, Davis (1975) reported no change in serum GH after TRH in immature ewe lambs. These differences are confusing but may be explained on the basis of species and/or age differences.

In humans, GH responses following TRH have been quite variable. In acromegalic humans, serum GH increased following TRH (Saito et al., 1971, Irie and Tsushima, 1972, Cryder et al., 1973 and Faglia et al., 1973). Also, serum GH increased following TRH in patients with depression (Maeda et al., 1975) or anorexia nervosa (Maeda et al., 1976). However, in normal healthy humans the serum GH response to TRH is confusing. Torjesen et al. (1973) reported increased serum GH following TRH administration to women, but not men. In contrast, Anderson et al. (1971), Saito et al. (1971), Irie and Tsushima (1972) and Maeda et al. (1975) reported no change in serum GH following TRH in normal healthy humans.

In view of the ability of TRH to stimulate prolactin and GH release, this hypothalamic releasing factor has been tested for galactopoietic activity. For example, Tyson et al. (1972) reported breast engorgement and bilateral milk letdown in women approximately 2.5 hours after TRH injection. Prior to TRH administration, breast engorgement did not occur in any of the breast-feeding mothers. Convey et al.

(1973) reported an increase of 0.66 kg/cow/day in milk yield in 20 cows receiving TRH. In contrast, Kelley et al. (1973) and Schams et al. (1974) observed no increase in milk production following TRH in lactating cows although few cows were involved in each experiment and doses of TRH were different. In addition, Adams et al. (1973) detected no differences in litter weight gain in rats after TRH administration to lactating mothers when compared with litter weight gain in control rats. However, when TRH administration began prior to parturition in cattle, a 10 to 30 percent increase in milk yield occurred during the first 20 days of lactation when compared with the same period for the previous year (Schams et al., 1974).

Seasonal Effects on Serum Prolactin and Growth Hormone

Several researchers have reported a seasonal effect on serum prolactin concentrations in cattle (Koprowski and Tucker, 1973, Schams and Reinhardt, 1974, Thatcher, 1974, Tucker et al., 1974 and Buttle and Forsyth, 1976) and goats (Buttle, 1974 and Hart, 1975). Serum prolactin is highest in summer and lowest in winter months in these species. The available data suggest that illumination and temperatures are important factors in the manifestation of seasonal cyclicity of serum prolactin.

Relkin (1972) observed the effects of diurnal lighting, constant lighting or constant darkness on serum and pituitary prolactin in rats. Constant lighting for 7 days resulted in a 67 percent increase in pituitary prolactin when compared with diurnal lighting; on the other hand, serum prolactin was suppressed 25 percent. Constant darkness resulted in increased serum prolactin, but decreased pituitary prolactin. In contrast, Reiter (1975) found in hamsters a 50 percent decrease in serum

prolactin after 56 days of near constant darkness (light: dark cycles of 1 hour light and 23 hours darkness) when compared with light: dark cycles of 14 hours light and 10 hours darkness.

In cattle, shortening of the photoperiod from 16 to 8 hours over a 12-week interval decreased serum prolactin from a maximum of 57 ng/ml to a minimum of 8 ng/ml. In contrast, lengthening of the photoperiod from 8 to 16 hours of light increased serum prolactin from 25 to 100 ng/ml (Bourne and Tucker, 1975). In rams, serum prolactin concentrations were 16 times greater in rams exposed to 16 hours of daylight as compared with rams exposed to 8 hours of daylight (Pelletier, 1973). Furthermore, Forbes et al. (1975) found an increase in serum prolactin following exposure of castrated male lambs to 16 hours of daylight as compared with 12 hours of daylight in control lambs.

Temperature has also been shown to play a role in the regulation of prolactin in several species. Mueller et al. (1974) reported a 5- to 10-fold increase in serum prolactin following exposure of male rats to 40 C for 30 minutes. Exposure of rats to 4 C for 1 to 2 hours resulted in a significant decrease in serum prolactin when compared with control rats maintained at 24 C. In contrast, Jobin et al. (1975) reported an increase in serum prolactin in rats after exposure to 5 C for 32 days. These data are in direct conflict and further research is needed to clarify these results.

In heifer calves, a reduction of ambient temperature from 21 to 10 C over a 4-hour period decreased serum prolactin from 13 to 4 ng/ml (Wettemann and Tucker, 1974). When the temperature was increased from 21 to 27 C over a 3-hour period, serum prolactin increased from 8 to 22 ng/ml. Also, chronic exposure to 10 or 27 C for 5 days decreased or

increased serum prolactin, respectively, when compared with control values (21 C for 5 days). This early report was confirmed in a second set of experiments by the same authors (Tucker and Wettemann, 1976) using more extreme temperatures. In addition, the expected release of prolactin into serum following TRH administration was suppressed at 4.5 C.

Mueller et al. (1973) reported that plasma GH concentrations were decreased by 70 percent following exposure of rats to 40 C for 1 hour. In contrast, plasma GH concentrations were not significantly altered following exposure of rats to 4 C for 1 or 2 hours. Tucker and Wettemann (1976) found no significant differences in serum GH in heifer calves exposed to 4.5, 21 or 32 C for nine days although GH tended to increase with increasing temperatures. Serum GH concentrations in lactating cows (Koprowski and Tucker, 1973) or bulls (Tucker et al., 1974) were not affected by the season of the year.

Prolactin and Growth Hormone Prior to Puberty

Serum and pituitary prolactin increase markedly with the onset of puberty in rats. For example, in prepubertal female rats Minaguchi et al. (1968) reported uniformly low pituitary prolactin values at 21, 26 and 31 days of age. However, pituitary prolactin content markedly increased shortly after the onset of puberty. Voogt et al. (1970) also found low pituitary prolactin values in prepubertal female rats prior to vaginal opening. In addition, serum prolactin concentrations were low prior to vaginal opening, but increased markedly on the day of vaginal opening. Also, serum prolactin concentrations were reduced in

prepubertal male rats prior to the beginning of sexual development (Negro-Vilar et al., 1973, Dohler and Wuttke, 1974 and Dohler and Wuttke, 1975).

In male lambs, Ravault and Courot (1975) observed relatively stable serum prolactin concentrations from 4 to 9 weeks of age. A rapid increase in serum prolactin was noted at 10 to 12 weeks of age which occurred simultaneously with the beginning of a rapid increase in testicular weight and spermatogenic activity. Sinha and Tucker (1969) reported that pituitary prolactin content increased 333 percent between birth and 3 months of age in heifers with maximum values at 9 months which synchronized with changes in mammary development.

Armstrong and Hansel (1956) observed higher pituitary GH content in heifers at birth to one and 16 weeks of age when compared with postpubertal heifers. Similarly, in bull calves, pituitary GH content was greater at 2 and 4 months of age as compared with postpubertal values (Purchas et al., 1970) and these increased pituitary concentrations in prepubertal cattle probably reflected increased synthesis and release of GH because plasma GH concentrations were higher at birth but decreased to relatively stable values between 2 and 12 months of age.

Prolactin and Growth Hormone During the Estrous Cycle

Exogenous estrogen and progesterone enhance pituitary and serum prolactin concentrations. Reece and Turner (1937) reported that exogenous estrogen increased pituitary prolactin content in rats, and Nicoll and Meites (1962) found that estrogen increased prolactin release from rat pituitary explants *in vitro*. Chen and Meites (1970) reported that exogenous estrogen increased pituitary and serum prolactin

concentrations in ovariectomized rats. Pituitary and serum prolactin concentrations were greater in rats at proestrus and estrus when compared with diestrus (Sar and Meites, 1967, Amenomori et al., 1970, Voogt et al., 1970 and Ieiri et al., 1971) which was coincidental with increased plasma estrogen concentrations (Yoshinaga et al., 1969). The mechanism of action involved in increased serum and pituitary prolactin concentrations associated with increased plasma estrogen concentrations is unknown. However, hypothalami from rats killed during proestrus and estrus contained significantly less prolactin-inhibiting factor (PIF) than hypothalami taken from diestrus rats (Ratner and Meites, 1964 and Sar and Meites, 1967). These data suggest that estrogen suppresses PIF synthesis and/or secretion in the hypothalamus.

In the ewe, Reeves et al. (1970) reported significantly higher serum prolactin concentrations at proestrus and first day of estrus when compared with other stages of the estrous cycle. Bryant et al. (1971) and Davis et al. (1971) also reported increased serum prolactin concentrations in ewes on the day of estrus.

Koprowski and Tucker (1973) and Wettemann and Hafs (1973) found no difference in serum prolactin concentrations at various stages of the estrous cycle in lactating cows or in heifers. In contrast, increased pituitary prolactin concentrations have been reported for cattle during proestrus and estrus (Sinha and Tucker, 1969). Also, Swanson et al. (1972) reported increased serum prolactin concentrations around estrus. These data are confusing but the more extensive study by Koprowski and Tucker (1973) would seem to indicate that serum prolactin concentrations are variable during the estrous cycle but there is no particular pattern associated with different stages of the cycle.

Although serum prolactin was suppressed slightly during infusion of 17β-estradiol in cows, this hormone increased following cessation of the infusion (Schams and Karg, 1972). In contrast, Beck et al. (1976) found no differences in serum prolactin in ovarietomized heifers with or without estrogen implants. These authors suggested that estrogen did not influence serum prolactin when present in blood at concentrations comparable to those found during the normal estrous cycle.

As reported above, serum prolactin concentrations in many species are diminished during the luteal phase of the cycle when serum progesterone concentrations are increased. Reece and Bivens (1942) measured pituitary prolactin content in ovariectomized rats following estrogen, progesterone, and estrogen-progesterone injections. Progesterone increased prolactin content of the pituitary; however, estrogen was more effective in augmenting the prolactin content. Estrogen-progesterone augmentation of pituitary prolactin was intermediate between progesterone and estrogen in effectiveness. Sar and Meites (1968) reported that progesterone increased pituitary prolactin content by 51 percent over controls in ovariectomized rats. Hypothalamic PIF content was suppressed in the progesterone injected rats as compared with controls. Chen and Meites (1970) also reported increased pituitary and serum prolactin concentrations following progesterone injections in rats. These data suggest that progesterone may suppress PIF and thus increase pituitary and serum prolactin concentrations.

In bulls, infusion of 5 to 80 mg of progesterone during a 1-hour period resulted in as much as a 20-fold increase in serum prolactin (Schams et al., 1974). However, in ovariectomized heifers with a

progesterone pessary, serum prolactin concentrations were not different from concentrations in control heifers (Beck et al., 1976).

The physiological role of prolactin during the estrous cycle is still unknown in many species. Although serum prolactin concentrations are increased at proestrus and estrus, blockage of the pre-ovulatory prolactin surge with ergot drugs did not prevent normal ovulation from occurring in ewes (Kann and Denamur, 1974), nor did it affect normal corpora lutea function or estrous cycle length (Niswender, 1974).

Changes in estrogen concentrations may also influence GH concentrations. Serum and pituitary GH concentrations were greater at proestrus and estrus in rats (Dickerman, 1971) and mice (Sinha et al., 1972) when compared with diestrus. In contrast, Ieiri et al. (1971) reported that GH synthesis and release did not change significantly during the estrous cycle in rats. These data are confusing and further research is needed to clarify the relationship between estrogen and GH during the estrous cycle in rats. In humans, increased GH concentrations were noted during the ovulatory and pre-menstrual phases in women (Spellacy et al., 1969).

Koprowski and Tucker (1973) reported increased serum GH during estrus in lactating cows. Administration of diethylstilbesterol increased serum GH in steers (Trenkle, 1970) and rats (Lloyd et at., 1973). However, in ovariectomized heifers, Beck et al. (1976) found no differences in serum GH between heifers with and without estrogen implants.

Davis and Borger (1974) reported that the injection of progesterone appeared to enhance the secretion of GH in ovariectomized ewes.

But in ovariectomized heifers, Beck et al. (1976) reported that serum GH concentrations were not significantly changed by progesterone administration.

Prolactin and Growth Hormone During Pregnancy

The function of prolactin during pregnancy in cattle is unknown. Wettemann and Hafs (1971) found that serum prolactin did not change significantly during the first 75 days of pregnancy. Although serum prolactin concentrations were not significantly different at 90, 180 and 260 days of gestation, serum prolactin tended to increase at the third trimester of pregnancy (Oxender et al., 1972). Furthermore, numerous researchers have observed increases in pituitary and serum prolactin concentrations shortly before parturition which may be associated with initiation of lactation.

Bates et al. (1935) and Reece and Turner (1937a) reported that pituitary prolactin content increased during late pregnancy in cattle. Johke et al. (1971) found that serum prolactin ranged from 8.3 to 19.6 ng/ml during 4 to 55 days prepartum in cows. However, on the day before parturition, serum prolactin increased to a maximum of 137 ng/ml, but it declined shortly after parturition. Schams and Karg (1970) and Ingalls et al. (1973) also observed increased serum prolactin just prior to parturition in cows and heifers.

In the ewe, McNeilly (1971) observed increased serum prolactin concentrations during the last third trimester of pregnancy. In contrast, Arai and Lee (1967) reported a gradual decline in prolactin during advancing pregnancy. Davis et al. (1971) also reported decreasing or low serum prolactin in ewes during advancing pregnancy, but serum

prolactin began to increase gradually 3 to 5 weeks prior to parturition with a dramatic increase 3 days prior to parturition. Burd et al. (1976) also reported a dramatic increase in serum prolactin at parturition. In the goat, serum prolactin increased just prior to parturition (Johke et al., 1971). Although these data are confusing, perhaps because of confounding seasonal effects, the bulk of the data indicate that serum prolactin concentrations increased during late pregnancy.

Serum GH concentrations did not change significantly during pregnancy in cows (Oxender et al., 1972). Ingalls et al. (1973) found no change in serum GH for the interval 26 to 9 days before parturition. However, serum GH increased as parturition neared and was maximal at parturition. In contrast, Koprowski and Tucker (1973) reported a linear increase, although quantatively small, in serum GH with advancing pregnancy. Plasma GH was relatively low in the ewe throughout pregnancy (Bassett et al., 1969). Schalch and Reichlin (1966) and Dickerman (1971) observed no change in serum GH concentrations throughout pregnancy in the rat. Collectively, these data suggest that serum GH concentrations remain relatively stable throughout pregnancy but may increase around parturition.

Hypothalamic Control of Prolactin and Growth Hormone Secretion

Regulation of prolactin secretion is exerted mainly via the hypothalamus, and this appears to involve the actions of PIF, a possible prolactin-releasing factor (PRF), catecholamines, serotonin or perhaps other biogenic amines- all produced in the hypothalamus (Meites and Clemens, 1972). Early studies by Everett (1954) and Nikitovitch-Winer and Everett (1958) indicated that transplantation of the pituitary

underneath the kidney capsule resulted in continuous and increased prolactin secretion, as indicated by prolonged maintenance of luteal function in rats. Transplantation of 1 to 4 heterologous anterior pituitaries underneath the kidney capsule of female hypophysectomized-ovariectomized rats, resulted in continuous prolactin release during 10 weeks (Chen et al., 1970). Rats with no pituitary grafts had barely detectable concentrations of serum prolactin while increasing the number of pituitary transplants per kidney increased serum prolactin concentrations. These observations were the initial bases for postulating the long-term control of prolactin secretion by the hypothalamus was inhibiting, and that the hypothalamus secretes a PIF.

Placement of bilateral lesions in the median eminence, or anterior or posterior hypothalamus of ovariectomized or intact rats resulted in significant increase of serum prolactin above control values (Chen et al., 1970 and Welsch et al., 1971). This suggests that all of these areas participate in regulation of prolactin secretion. Halasz et al. (1962) have defined this region as the "hypophysiotropic area" and it is believed to control basal secretion of anterior pituitary hormones. Transection of the hypophysial stalk interrupts anterior pituitary blood supply from the hypothalamus. Kanematsu and Sawyer (1973) observed increased plasma prolactin after hypophysial stalk section in rats and since prolactin secretion is enhanced when the pituitary is transplanted to the kidney or following hypothalamic lesions, this further substantiated the presence of a hypothalamic inhibiting factor.

Talwalker et al. (1963) reported decreased prolactin release from rat pituitary incubates after addition of crude hypothalamic

extracts. Hypothalamic extracts from sheep, cattle, pigs and humans also were demonstrated to inhibit release of prolactin (Schally et al., 1967). Infusion of hypothalamic extracts into pituitary portal vessels decreased plasma prolactin concentrations in rats while increasing plasma concentrations of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (Kamberi et al., 1971). In addition, the prolactin responses to hypothalamic extracts were related to the concentration of the extract in a negative-dose response manner. Takahara et al. (1974) observed suppressed serum prolactin following the infusion of a purified porcine PIF preparation via a hypophysial portal vessel in the rat. This purified PIF preparation contained a high content of catecholamines.

The mechanism of action of PIF is still unknown. Ca^{++} ions have been shown to be essential for prolactin release from the pituitary *in vitro* (Parsons, 1969). Nicoll (1971) suggested that PIF acts on the prolactin secreting cell membrane to inhibit Ca^{++} influx. Parsons (1969) suggested that the cell depolarizes spontaneously when it is freed from hypothalamic influence, with a resultant increase in Ca^{++} entry into the cell and consequent release of secretory granules. Thus, PIF may act by preventing spontaneous depolarization of the cells which secrete prolactin.

Kamberi et al. (1971) reported decreased serum prolactin following the injection of dopamine into the third ventricle of the rat. Serum prolactin was suppressed by 30, 53 and 58 percent at 10, 20 and 30 minutes, respectively, following dopamine injections when compared with pre-injection prolactin concentrations. However, by 120 minutes post-injection, serum prolactin was suppressed by only 8 percent. Epinephrine and norepinephrine also suppressed serum prolactin following

intraventricular injection. In contrast, dopamine, epinephrine and norepinephrine, perfused into a hypophysial portal vein, failed to alter serum prolactin. These findings suggest that neither dopamine, epinephrine nor norepinephrine affect prolactin release by direct action on the anterior pituitary but indirectly through the hypothalamic-hypophysial complex. In agreement with these results were the findings that systemic administration of L-dopa suppressed serum prolactin concentrations, increased hypothalmic PIF content and resulted in prolactin-inhibiting activity in the serum of rats (Lu and Meites, 1972).

Shaar and Clemens (1974) observed that rat hypothalamic extracts significantly inhibited pituitary prolactin release in vitro. These authors further indicated that the prolactin inhibiting activity of hypothalamic extracts can be totally accounted for by the amount of endogenous catecholamines in the hypothalamus.

The evidence for the possible existence of PRF is much less convincing than that for PIF. Several researchers have reported the possible existence of PRF (Meites et al., 1960, Mishkinsky et al., 1968 and Nicoll et al., 1970). However, various other researchers have cast doubts on the existence of PRF.

In contrast to the inhibiting role of catecholamines on prolactin secretion, the biogenic amine, serotonin, has been shown to increase serum prolactin. Kamberi et al. (1971) reported that injection of serotonin and melatonin into the third ventricle of the brain of male rats stimulated release of prolactin. In addition, neither melatonin nor serotonin infusion into the anterior pituitary resulted in a significant alteration of plasma prolactin. Lu and Meites (1972) reported increased serum prolactin following the injection of

tryptophan, 5-hydroxy-tryptophan or melatonin into rats. Serotonin failed to alter serum prolactin but this may be related to its inability to pass the blood-brain barrier.

The pineal gland has been implicated recently in the regulation of prolactin secretion (Reiter, 1974 and Blask and Reiter, 1975). White et al. (1974) reported that the pineal gland can act as a supplemental source of hypothalamic-releasing hormones. As reported previously, the hypothalamus contains TRH which can increase serum prolactin when given exogenously. Whether TRH plays a role in the regulation of prolactin under normal physiological conditions remains to be seen.

The regulation of serum GH by the hypothalamus is less clearly understood than for prolactin. The secretion of GH from the pituitary is under the control of a hypothalamic GH-releasing factor (GRF) and GH-inhibiting factor (GIF) or somatostatin (SRIF). Deuben and Meites (1964) demonstrated for the first time that rat hypothalamic extracts promoted the released of GH from 6-day cultured rat pituitary glands. These results were confirmed by Schally et al. (1965) who reported that the addition of highly purified porcine GRH to rat anterior pituitaries incubated in vitro stimulated the synthesis and release of GH.

GH-inhibiting factor or somatostatin has been shown by several researchers to inhibit GH release from the pituitary. Krulich et al. (1968) and Dhariwal et al. (1969) reported the separation of GIF from other hypothalamic factors. Stachura et al. (1972) observed that ovine hypothalamic fractions purified on Sephadex G-25 almost completely inhibited synthesis and release of GH in a rat pituitary in vitro system. Similarly, Brazeau et al. (1973) determined that a purified extract from sheep hypothalami inhibited GH release in rats. Brazeau

et al. (1974) also reported that SRIF had an inhibiting effect on GH release induced by isoprenalin or chlorpromazine in rats.

In general, catecholamines stimulate GH secretion while serotonin has no effect (Wilson, 1974). Mims et al. (1975) observed increased GH secretion following oral administration of L-dopa in humans. Similarly, serum GH concentrations were increased in Rhesus monkeys following infusion of L-dopa (Chambers and Brown, 1976).

MATERIALS AND METHODS

A. Animals

Prepubertal heifers, 4 to 5 months of age and weighing 125 to 148 kg, were used in the first four experiments. In the fifth experiment, heifers exhibiting normal estrous cycles were used. Heifers that were either 3, 6 or 9 months pregnant were used in the sixth experiment. In the seventh experiment, primiparous and multiparous lactating cows were used. Prepubertal, cycling and pregnant heifers were maintained under loose housing conditions prior to start of experimentation with free access to feed and water. Lactating cows were maintained in stanchions with free access to water and were fed according to recommended practices for milk production. All cattle were of the Holstein breed.

B. Cannulation, Blood Sampling and Processing Procedures

All animals were fitted with indwelling polyvinyl jugular cannulae (V-10 tubing, Bolab, Inc., Derry, N.H.) on the day prior to experimentation in experiments 1, 2, 3, 4, 6 and 7. In experiment 5, heifers were cannulated on the morning of estrus or one day prior to the first TRH injection on day 15 of the estrous cycle. Cannula were flushed with 3.5 percent sodium citrate containing 50 percent dextrose

dissolved in 0.85 percent NaCl solution. This solution was used to prevent coagulation of blood and thus maintain functionality of the cannula throughout the experimental period.

Blood samples were collected at various intervals, depending on experimental protocol, before and after TRH injections. To condition animals to the blood sampling procedure and thereby establish stable baseline values for prolactin (Raud et al., 1971 and Tucker, 1971), blood samples were collected and discarded (not assayed for hormones) at 15-minute intervals for 2 to 3 hours prior to start of experimentation. Thereafter, blood samples were collected for hormone assay. Sera were prepared from the blood samples and stored at -20 C until assayed for prolactin and GH.

C. Hormone Assays

Serum hormone concentrations were quantified by double antibody radioimmunoassay (RIA) procedures as previously described for prolactin (Tucker, 1971 and Koprowski and Tucker, 1971) and GH (Purchas et al., 1970).

D. Experimental Procedures and Objectives

Experiment 1. Repeatability of Changes in Serum Prolactin and GH Concentrations in Response to a Given Dose of TRH

Experimental Design.--Eight heifers received one intravenous (IV) injection of 10 µg TRH in 10 ml 0.85 percent NaCl on days 1, 2, 4 and 8. Heifers were selected on the basis of uniformity in age and weight. During experimentation, heifers were housed in individual tie stalls. Collections of blood and IV injections were via polyvinyl

jugular cannulae. Cannulae were opened at 1000 hours with blood samples collected for hormones assay (table 1) starting at 1230 hours.

Table 1.--Time of sampling relative to thyrotropin releasing hormone (TRH) administration

Experiment	Pre-treatment[a]	Post-treatment[a]
1	-30, -15, -10, -5, 0	4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 45, 60
2 and 3	-30, -25, -20, -15, -10, -5, 0	4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30
4	-15, -10, -5, 0	4, 6, 8, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120
5 and 6	-15, -10, -5, 0	4, 6, 8, 10, 15, 20, 25, 30
7	-30, -15, -5, 0	4, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30

[a]Minutes relative to TRH administration

Experiment 2. Effects of Various Doses of TRH on Serum Prolactin and GH

Experimental Design.--The effects of various doses of TRH on serum prolactin and GH were measured in six heifers. Each heifer received IV injections in a sequence determined at random of either 10 ml 0.85 percent NaCl or 5, 10, 25, 50 or 100 µg TRH in 10 ml 0.85 percent NaCl during six consecutive days. However, only one heifer received a given dose on any day. Heifers were selected, housed, bled and injected as described previously in experiment 1. Cannulae were

opened at 0930 or 1030 hours with blood samples collected for hormone assay (table 1) starting at 1130 or 1230 hours.

Experiment 3. Effects of Various Doses of TRH Via Different Routes of Administration on Serum Prolactin and GH

Experimental Design.--The six heifers used in experiment 2 were employed subsequently to determine the effects of route of administration of TRH on serum prolactin and GH concentrations during 30 minutes after TRH was given. An interval of 24 hours was allowed between the last injections of TRH or saline in experiment 2 and the start of experiment 3. On day one, two heifers were injected IV, two were injected intramuscularly (IM) and two were injected subcutaneously (SC) with one ml of 0.85 percent NaCl. Between days 2 and 7, each heifer received, in a sequence determined at random, either 10 or 25 μg TRH in 1 ml 0.85 percent NaCl injected IV, Im or SC. Thus, each heifer received each dose of TRH (10 and 25 μg) via each route of administration (IV, IM and SC). Each combination of dose and route of administration was given only once on a given day. Injections of NaCl, via a different route of administration for each heifer, were repeated on day 8. However, since only 2 days were allowed for NaCl injections, each heifer received NaCl via only two of the three possible routes of administration. All other conditions and procedures were as stated in experiment 2.

Experiment 4. To Determine the Effects of an Increased Dose of TRH Via Different Routes of Administration on Serum Prolactin and GH

Experimental Design.--Results from experiment 3 indicated that serum prolactin may not have reached maximum concentrations during 30 minutes after TRH administration, nor were the doses sufficiently great to achieve concentrations of prolactin or GH which were as great as those in experiment 2. Thus, four heifers were injected IV or SC in random sequence during four consecutive days with either 1 ml 0.85 percent NaCl or 50 µg TRH in 1 ml 0.85 percent NaCl. However, no combination was administered more than once on a given day. Blood samples were collected for 2 hours after treatment (table 1). All other conditions and procedures were as stated in experiment 2.

Experiment 5. Effect of TRH on Serum Prolactin and GH During the Estrous Cycle

Experimental Design.--Six heifers, exhibiting normal estrous cycles, received IV injections of 33 µg TRH/100 kg body weight in a randomized, complete-block-repeat-measurement-design on days 0 (estrus), 2, 4, 7, 15 and 18 of the estrous cycle. Three heifers received their initial injection of TRH on day 0 and the other three were injected initially on day 15 of the estrous cycle. All heifers were observed for estrus during two estrous cycles prior to the start of experimentation. During these two estrous cycles, all heifers exhibited cycles ranging from 19 to 22 days in length. This was the criterion used for deciding that the estrous cycles were "normal". Heifers were observed for estrus at 0700 and 1700 hours for a 30-minute period after being moved to an exercise lot. During all other times of the experimental

period, heifers were housed in individual tie stalls. Collection of blood and IV injections were via polyvinyl jugular cannulae. Cannulae were opened at 0930 hours with blood samples collected for hormone assay (table 1) at 1145 hours.

Experiment 6. Effect of TRH on Serum Prolactin and GH During Pregnancy

Experimental Design.--Twenty-seven pregnant heifers received IV injections of 33 µg TRH/100 kg body weight according to a 3 x 3 factorial design. TRH was injected at either 3, 6 or 9 months of pregnancy during either November, April or July. Nine heifers received TRH during November, April or July with three heifers each in either the first, second or third trimester of pregnancy. However, only two heifers in the first trimester of pregnancy received TRH during July due to loss of a cannula from one heifer. Thus, each heifer received only one injection of TRH during the experimental period. All heifers were diagnosed pregnant on the basis of rectal palpation by a veterinarian, with the last breeding date used as the date of conception. Collections of blood and IV injections were via polyvinyl jugular cannulae. Cannulae were opened 2 to 3 hours prior to start of experimentation with blood samples collected for hormone assay as shown in table 1.

Experiment 7. Effect of TRH on Serum Prolactin and GH During Lactation

Experimental Design.--Sixteen primiparous or multiparous lactating cows received IV injections of 33 µg TRH/100 kg body weight at 2, 4, 6, 8 and 10 months of lactation. Each cow received TRH on two consecutive days at each stage of lactation to determine if there were

any carryover effects on serum prolactin and GH following TRH. Cows were started on the experiment in groups of four. Groups were started in May, September, October and November. Although five cows were dried-off prior to the 10-month stage of lactation, their data are included in the analysis. Collections of blood and IV injections were via polyvinyl jugular cannulae. Cannulae were opened 2 to 3 hours prior to start of experimentation with blood samples collected for hormone assay as shown in table 1.

E. Statistical Methods

Average baseline concentrations (ng/ml) of prolactin and GH prior to TRH were calculated for each animal on each day. This value was subtracted, within animal, from subsequent corresponding hormone concentrations measured at each post-treatment sampling time. These adjusted values (ng/ml) were plotted against time (minutes) and a third degree least squares polynomial curve was computed. The area under this hormone response curve was integrated and expressed as ng ml^{-1} minutes. In addition, average hormone concentrations at the post-treatment peak and average time (minutes) required to achieve peak hormone concentrations were calculated for each experiment.

In experiments 1, 2, 3 and 4, data were analyzed by analysis of variance, regression analysis and comparisons of means of areas under individual hormone response curves. Dunnett's procedure was used for unbalanced data in experiment 3 since I was primarily interested in comparing IM or SC route of administration of NaCl or TRH with IV route of administration. Tukey's statistic was used to compare all means (balanced data) in experiments 1, 2 and 4 (Kirk, 1968). In

experiment 5, analysis of variance was used. Analysis of variance and Tukey's statistic were used to compare means of the areas under individual hormone response curves in experiment 6. In experiment 7, analysis of variance and analysis of covariance were used. Also used were preselected orthogonal contrasts among means of areas under individual hormone response curves.

RESULTS AND DISCUSSION

Experiment 1. Repeatability of changes in serum prolactin and GH concentrations in response to a given dose of TRH

This experiment was designed to determine repeatability of changes in serum prolactin and GH concentrations in response to a given dose of TRH on days 1, 2, 4 and 8.

Serum prolactin concentrations, averaged across the four days of the experiment, increased from a baseline value of 16 ng/ml to a peak of 81 ng/ml at 7 minutes after IV administration of 10 μg TRH. Magnitude of increase in serum prolactin concentrations, as measured by areas under the response curves (ng ml^{-1}min), after administration of TRH averaged 1,393, 1,532, 1,381 and 1,585 on days 1, 2, 4 and 8, respectively. These means were not significantly different from each other ($P>.05$). These results extend to prepubertal female calves the finding that administration of TRH releases prolactin in mature bulls (Convey et al., 1973 and Tucker et al., 1974) and cows (Schams, 1972, Convey et al., 1973 and Kelly et al., 1973).

Repeatability coefficients of average baseline concentrations for prolactin (within heifer, among four days of sampling) was .27, whereas repeatability of the area under the prolactin response curve after TRH administration was .61. The correlation coefficient (within heifers) between average serum prolactin concentrations before TRH and

maximum prolactin concentrations after TRH was .64 ($P<.01$). Thus, the amount of prolactin released from the pituitary following TRH is very closely related to basal or resting serum prolactin concentrations. This implies that the quantity of prolactin released following an exogenous stimulus such as TRH may be related to the rate of tonic release of prolactin from the pituitary. Wettemann and Tucker (1974) and Tucker and Wettemann (1976) also reported that greater concentrations of basal or resting serum prolactin were associated with greater peak heights after administration of TRH. In contrast, Koprowski and Tucker (1973) found a negative relationship between baseline or resting concentrations of serum prolactin and subsequent milking-induced releases of this hormone. These findings may suggest that milking- and TRH-induced releases of prolactin are acting through different mechanisms. Koprowski and Tucker (1973) reported a correlation coefficient of .36 ($P<.01$) between serum prolactin concentrations immediately after milking and milk yield. Thus, if serum prolactin concentrations following TRH administration are affected by baseline concentrations, possible use of serum prolactin concentrations following TRH administration as an indicator of potential milk producing ability is not very promising in prepubertal heifers.

Baseline serum GH concentrations prior to TRH averaged 8 ng/ml and increased to a maximum of 21 ng/ml at 10 minutes after TRH. These results extend to prepubertal female calves the finding that administration of TRH releases GH in mature bulls (Tucker et al., 1974) and cows (Convey et al., 1973).

The correlation coefficient between serum GH concentrations measured before TRH and maximum GH concentrations after TRH was .60

($P<.01$). The increase in serum GH concentrations after TRH averaged 148, 102, 102 and 142 ng ml^{-1}min on days 1, 2, 4 and 8, respectively. There were no significant differences among means ($P>.05$).

Repeatability coefficients for baseline serum GH concentrations and post-TRH response areas (calculated within heifers, among four days of sampling) were .37 and .35, respectively. Tucker et al. (1974) reported .30 repeatability estimate for basal or resting serum GH concentrations in mature bulls over a 10-day period. These results agree very closely with the repeatability estimate (.37) that I found in prepubertal heifers. In contrast, the repeatability estimate for serum GH response areas (.35) after TRH was only about one-half that for prolactin (.61). These results demonstrate that the pituitary is capable of releasing more prolactin than GH following TRH administration. This may be related to the different mechanisms controlling the releases of these two hormones since prolactin release is thought to be primarily under tonic inhibition and GH primarily under stimulatory control (Meites and Clemens, 1972 and Wilson, 1974).

Experiment 2. Effects of various doses of TRH on serum prolactin and GH

This experiment was designed to determine if varying the dose of TRH would affect serum prolactin and GH concentrations and if so to determine the doses where maximum hormone releases were attained.

Overall, baseline concentrations of prolactin in serum before TRH averaged 39 ng/ml. Injection of .85 percent NaCl did not affect ($P>.05$) serum prolactin concentrations. Increasing the dose of TRH from 5 to 100 μg increased peak serum prolactin concentrations from 143 to 277 ng/ml, but had little effect on time (8 to 12 min) required

to achieve these peaks (table 2). Doses of TRH and heifers affected ($P<.01$) the areas under the prolactin response curves. The quantity (response curve areas) of prolactin released increased linearly ($P<.01$) with increases in log of the dose of TRH. Although average prolactin response curve areas appeared to plateau between 50 and 100 µg TRH, the quadratic component of regression for these data was not significant ($P>.05$). Fell et al. (1973) and Noel et al. (1974) also reported a dose-response increase in serum prolactin concentrations following TRH administration in ewes and humans. In contrast, Convey et al. (1973) and Kelly et al. (1973) reported that serum prolactin concentrations were not clearly related to the dose of TRH administered in lactating cows. These differences may be due to more stable serum prolactin concentrations in calves than those found in lactating cows; thus, with greater endogenous variation the dose responsiveness to exogenous TRH may have been masked in those studies using lactating cows.

Although integrated areas under serum prolactin response curves increased with log of the dose of TRH, eventually a dose (50 µg) was reached where additional TRH (100 µg) did not cause a further increase in prolactin response curve areas. This dose may represent the maximum capacity of the anterior pituitary to release prolactin to a given stimulus. These results are in agreement with those published by Tucker et al. (1975) in which they suggested that a ceiling exists in postpubertal heifers and lactating cows for release of prolactin and GH in response to constant infusion or multiple injections of TRH. However, this ceiling does not necessarily represent the maximum capacity of the pituitary to release prolactin since Tucker et al. (1975) showed that stimultaneous application of a second heterologous stimulus such as

Table 2.--Serum prolactin (PRL) and growth hormone (GH) responses to various doses of thyrotropin releasing hormone (TRH)[a]

		Peak		
TRH (μg)	Hormone	Concentration (ng/ml)	Time to: (min)	Area[b] (ng ml^{-1} min)
0	PRL	---	---	-110 ± 128
(.85% NaCl)	GH	---	---	7 ± 15
5	PRL	145	12	1515 ± 602
	GH	11	5	53 ± 19
10	PRL	143	8	1729 ± 406
	GH	15	8	129 ± 45
25	PRL	211	10	3255 ± 833
	GH	29	11	355 ± 116
50	PRL	265	9	3820 ± 816
	GH	46	9	555 ± 137
100	PRL	277	10	3940 ± 448
	GH	42	10	667 ± 102

[a]Overall serum hormone concentrations (ng/ml) before TRH averaged 39 for PRL and 5 for GH.

[b]Integrated area of plot of hormone concentrations for 30 min after NaCl or TRH minus baselines before NaCl or TRH, respectively. Values are means ± standard errors (n=6).

milking or injection of prostaglandin $F_{2\alpha}$ would induce additional prolactin release. These data suggest that the pituitary may become refractory to TRH since the pituitary is still responsive to a second heterologous stimulus.

Serum GH concentrations before TRH administration averaged 5 ng/ml. Serum GH concentrations increased to peaks of 11 to 46 ng/ml following injections of 5 to 100 μg TRH (table 2). These peaks were attained 5 to 11 minutes after TRH was administered. Dosage of TRH and heifers affected ($P<.01$) the quantity of GH released, as measured by area under the response curve, but NaCl did not ($P>.05$). Furthermore, area under the GH response curve increased linearly ($P<.01$) with

increasing log of the TRH dose. Convey et al. (1973) also reported a dose-response increase in serum GH concentrations following TRH administration in lactating cows. Unlike prolactin release from the pituitary, these data indicate that the maximum dose (100 µg) of TRH did not maximally stimulate the pituitary to release GH. This submaximal stimulation of GH release from the pituitary following TRH administration is probably related to smaller amounts of GH, as compared with prolactin, that are released to a give dose of TRH. Thus, a larger dose of TRH is necessary for maximal stimulation of GH release.

Experiment 3. Effects of various doses of TRH via different routes of administration on serum prolactin and GH

This experiment was designed to determine the effects of TRH administrated via IV, IM or SC injection on serum prolactin and GH concentrations using different doses of TRH.

Serum prolactin concentrations averaged 22 ng/ml prior to administration of TRH. There was no prolactin response to the IV, IM or SC administration of NaCl ($P>.05$). Maximum serum prolactin concentrations after IV administration of 10 or 25 µg TRH occurred within 7 and 9 minutes, and averaged 145 and 174 ng/ml, respectively (table 3). Following IM or SC administration of TRH the peak heights were lower (48 to 107 ng/ml) and the time required to reach the peaks were longer (19 to 22 min) than those listed above for IV injections. Schams (1972) reported a longer lasting peak of serum prolactin concentrations following IM injections of TRH when compared with IV injections of TRH in cattle. He also reported a tendency for enhanced serum prolactin concentrations following increasing doses of TRH via IV administration.

Table 3.--Serum prolactin (PRL) responses to doses of thyrotropin releasing hormone (TRH) and routes of administration[a]

Dose of TRH (μg)	Route of Administration	Peak Concentration (ng/ml)	Peak Time to: (min)	Area[b] (ng ml^{-1} min)
0 (.85% NaCl)	IV	---	---	-34 ± 105
	IM	---	---	23 ± 228
	SC	---	---	142 ± 106
10	IV	145	7	1544 ± 398
	IM	48	19	167 ± 145
	SC	64	22	533 ± 309
25	IV	174	9	2170 ± 477
	IM	105	22	1322 ± 552
	SC	107	22	798 ± 253

[a]Overall serum PRL concentrations (ng/ml) before TRH averaged 22.

[b]Integrated area of plot of hormone concentrations for 30 min after NaCl or TRH minus baselines before NaCl or TRH, respectively. Values are means ± standard errors (n=4 for heifers receiving NaCl; n=6 for heifers receiving 10 or 25 μg TRH).

In total, these results suggest that IM and SC injected TRH is retained within the depot sites and released from these stores relatively slowly over a period of time. Therefore, serum concentrations of TRH immediately following IM and SC injections of TRH are less than those after IV injection, but these concentrations are maintained for a longer of time.

Doses of TRH, routes of administration and heifers affected ($P<.01$) areas under the serum prolactin response curve. In addition, there was a dose x route interaction ($P<.05$).

In comparison with NaCl controls, the serum prolactin response was greater after IV administration of 10 ($P<.05$) or 25 ($P<.01$) μg TRH.

Although mean prolactin response curve areas after 10 or 25 μg TRH administered IM or SC were numerically greater than control response areas, they were not significantly differently ($P>.05$). Areas under the prolactin response curve after 10 μg TRH injected IV were greater ($P<.05$) than that after IM administration of TRH. On the other hand, prolactin response areas after 10 μg TRH administered IM or SC were not different ($P>.05$) from each other. Nor were the areas under the prolactin response curve different ($P>.05$) after IV, IM or SC injections of 25 μg TRH (Figure 1.). Again, these data point out that more prolactin is released, although not always significantly greater, following IV administration of TRH, as compared with IM or SC injection, during a 30-minute post-TRH sampling period.

Overall, serum GH baseline concentrations averaged 7 ng/ml before TRH treatment. Injection of NaCl via any of the routes tested did not increase serum GH ($P>.05$). The means of maximum GH concentrations following IV, IM and SC injections of 10 μg TRH were 24, 14 and 11 ng/ml and they were attained at 7, 19 and 18 minutes, respectively (table 4). Following 25 μg TRH, serum GH peaks averaged 35, 25 and 14 ng/ml and occurred at 12, 14 and 22 minutes, respectively.

Doses of TRH, routes of administration, heifers ($P<.01$) and interaction of doses and routes ($P<.05$) affected areas under the serum GH response curves. Intravenous administration of 10 or 25 μg TRH increased serum GH response areas above controls ($P\simeq.05$); $P<.05$, respectively). However, neither IM or SC administration of 10 or 25 μg TRH increased ($P>.05$) the GH response curve area over that of NaCl controls. In addition, GH response areas after IV, IM or SC administration of 25 μg TRH did not differ from each other (Figure 2). These data show

Figure 1. Serum prolactin following IV, IM or SC administration of 25 µg TRH in prebpubertal heifers.

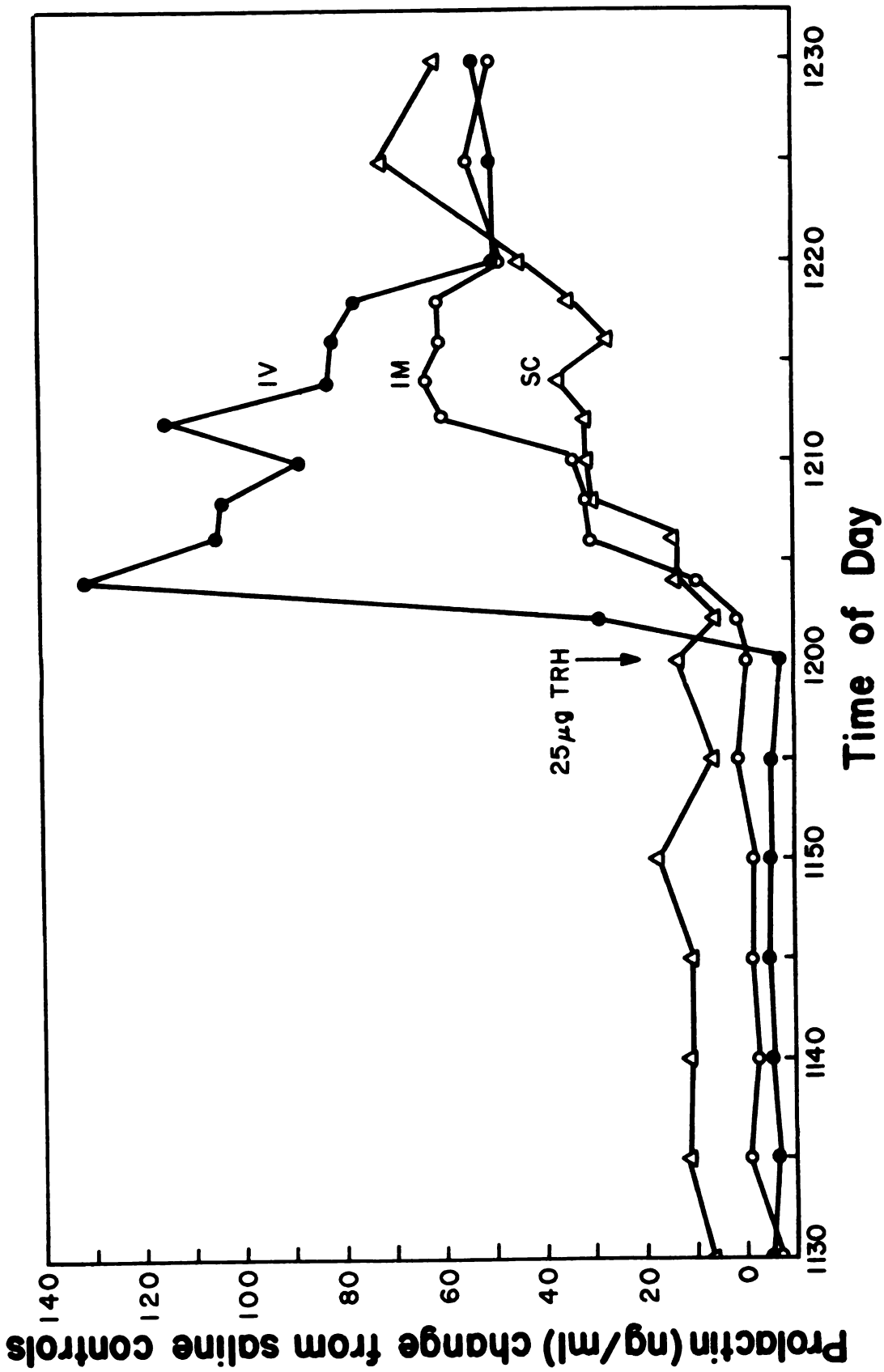

Prolactin (ng/ml) change from saline controls
140
120
100
80
60
40
20
0
1130
1140
1150
1200
1210
1220
1230
Time of Day
IV
IM
SC
25μg TRH

Table 4.--Serum growth hormone (GH) responses to doses of thyrotropin releasing hormone (TRH) and routes of administration[a]

Dose of TRH (μg)	Route of Administration	Peak Concentration (ng/ml)	Peak Time to: (min)	Area[b] (ng ml^{-1} min)
0 (.85% NaCl)	IV	---	---	-34 ± 38
	IM	---	---	12 ± 50
	SC	---	---	102 ± 74
10	IV	24	7	232 ± 47
	IM	14	19	82 ± 42
	SC	11	18	-23 ± 51
25	IV	35	12	326 ± 99
	IM	25	14	147 ± 87
	SC	14	22	115 ± 17

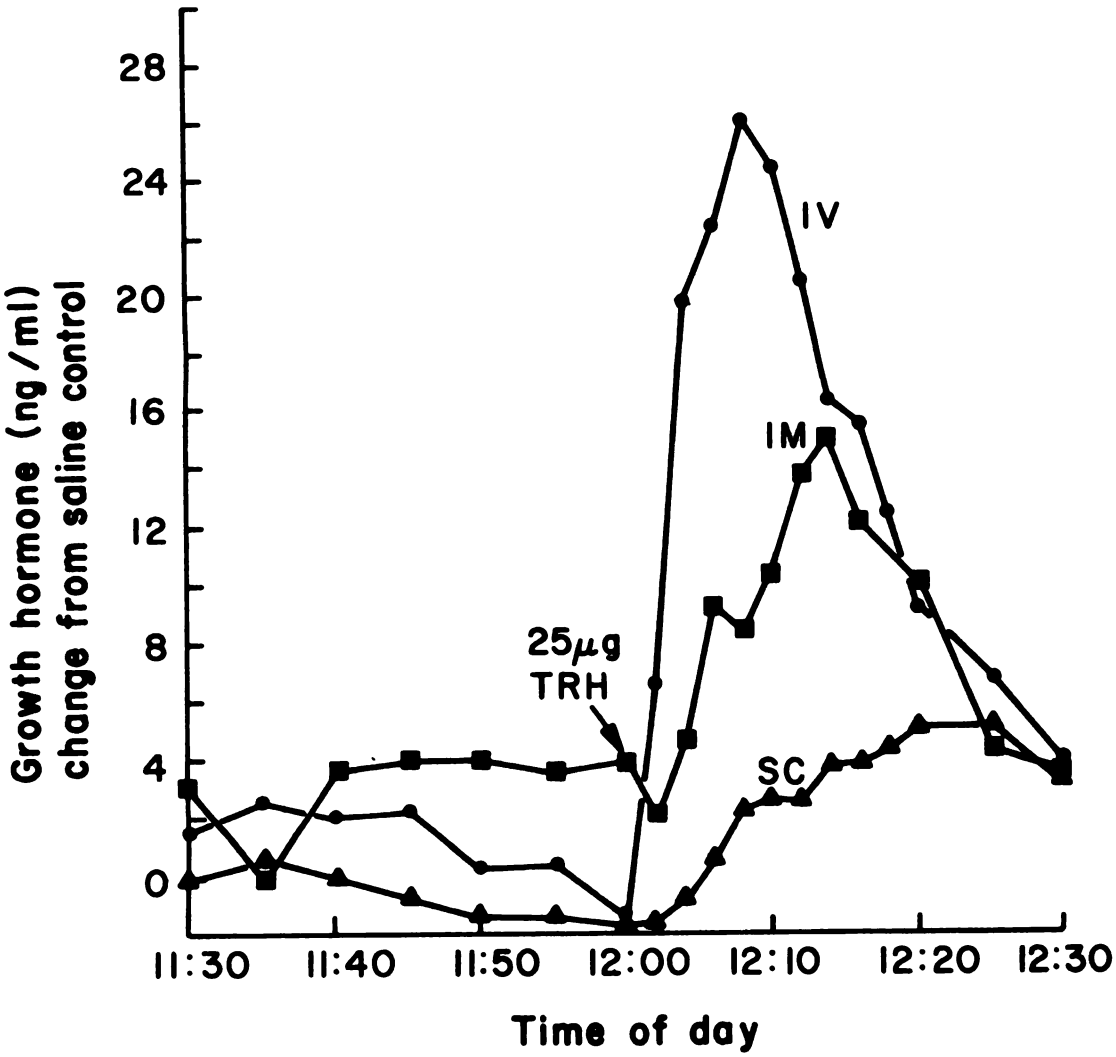
[a]Overall serum GH concentrations (ng/ml) before TRH averaged seven.

[b]Integrated area of plot of hormone concentrations for 30 min after NaCl or TRH minus baselines before NaCl or TRH, respectively. Values are means ± standard errors (n=4 for heifers receiving NaCl; n=6 for heifers receiving 10 or 25 μg TRH).

that more GH is released, although not always significantly greater, from the pituitary following IV administration of TRH when compared with IM or SC administration. As discussed previously in this section concerning release, IV administered TRH reaches the pituitary in greater concentrations earlier and results in a greater initial release of GH than after IM or SC injections of TRH.

The fact that serum GH response curve areas following IV administration of 25 μg TRH, as compared with IM or SC administration, were numerically but not statistically different can partially be attributed to heifer variation since heifers affected ($P<.01$) areas under the response curve.

Figure 2. Serum growth hormone following IV, IM or SC administration of 25 µg TRH in prepubertal heifers.


Growth hormone (ng/ml)
change from saline control
28
24
20
16
12
8
4
0
IV
IM
SC
25μg
TRH
11:30
11:40
11:50
12:00
12:10
12:20
12:30
Time of day

Experiment 4. Effects of an increased dose of TRH via different routes of administration on serum prolactin and GH

This experiment was designed to determine if increasing the dose of TRH to obtain near maximum hormone releases, as determined in a previous experiment, affected the total amount of prolactin and GH released over a 2-hour post-TRH sampling period.

Serum prolactin concentrations averaged 17 ng/ml before injection of 50 μg TRH. Maximum prolactin concentrations after IV and SC injections of TRH averaged 119 and 81 ng/ml, respectively, and these peaks were attained 7 and 46 minutes after TRH administration, respectively. Administration of TRH by IV or SC routes increased ($P<.01$) magnitude of prolactin release above that of NaCl controls (table 5), but there was no difference in area under the prolactin response curve ($P>.05$) between IV and SC routes of TRH administration. As in experiment 3, route of administration of TRH affected maximum serum prolactin concentrations and time required to achieve these peaks. However, when sufficient time is allowed following TRH administration for serum prolactin to return to pretreatment concentrations, the total prolactin response curve areas were not different after IV or SC administration of TRH. These results suggest that while route of administration affected the magnitude of response and time required to reach maximal concentrations of prolactin, the total amount of prolactin released following TRH injection is unaffected by route of administration. Therefore, when a prolonged release of prolactin is desired, the IM or SC route of administration of TRH should be the method of choice.

Table 5.--Serum prolactin (PRL) and growth hormone (GH) responses to doses of thyrotropin releasing hormone (TRH) and routes of administration[a]

	Route of Administration			
	PRL		GH	
Treatment	IV	SC	IV	SC
	ng ml^{-1} min			
.85% NaCl	-249 ± 252	464 ± 182	122 ± 92	45 ± 139
50 µg TRH	3639 ± 638	4198 ± 1390	504 ± 93	218 ± 247

[a]Area of plot of hormone concentrations for 120 min after NaCl or TRH minus baselines before NaCl or TRH, respectively. Values are means ± standard errors (n=4, except for GH after IV administration of 50 µg TRH where n=3).

Serum GH concentrations increased from 4 ng/ml before TRH to peaks of 28 and 17 ng/ml after IV or SC injections of 50 µg TRH, respectively. The time required to achieve these peaks was 8 and 28 minutes, respectively. One heifer did not reach maximal GH concentrations (16 ng/ml) until 70 minutes after IV injection of TRH. In the author's opinion this release was not associated with the TRH injection; thus, GH data after IV administration of 50 µg TRH for this heifer were excluded. The area under the GH response curves after IV injection of 50 µg TRH was greater ($P<.05$) than that for NaCl controls (table 5); whereas, the response to SC injection of 50 µg TRH was not different from controls ($P>.05$). These data suggest that high serum concentrations of exogenous TRH following IV administration will result in a greater total release of GH from the pituitary over a 2-hour period, even though a more sustained release of GH is obtained following SC administration of TRH.

Overall, results from experiments 3 and 4 demonstrate that serum prolactin and GH concentrations increase more rapidly and decline more precipitously following IV administration of TRH when compared with either IM or SC administration of TRH. In addition, maximum serum prolactin and GH concentrations are greater after IV administration of TRH, but increased serum prolactin and GH concentrations are sustained for longer periods of time following IM or SC administration of TRH.

Experiment 5. Effect of TRH on serum prolactin and GH during the estrous cycle

This experiment was designed to determine if different stages of the estrous cycle would affect prolactin and GH release from the pituitary following IV administration of TRH.

Overall, baseline concentrations of prolactin in serum before TRH averaged 6 ng/ml. Maximum serum prolactin concentrations after TRH ranged from 52 to 81 ng/ml and time required to attain these maximal values ranged from 10 to 17 minutes (table 6). Days of the estrous cycle did not affect ($P>.05$) the quantity of prolactin released, as measured by area under the response curve. Although prolactin released in response to injections of TRH at various days of the estrous cycle were not significantly different, maximum concentrations after TRH, pre-TRH concentrations and areas under the prolactin response curve at days 2 and 4 of the estrous cycle tended to be less than those at other stages of the estrous cycle. These suppressed prolactin responses may be related to low serum estrogen and progesterone concentrations. Serum estrogen and progesterone concentrations are known to be decreased at this time when compared with other stages of the estrous cycle (Wettemann et al., 1972). The concept that low serum estrogen and/or

Table 6.--Serum prolactin response to thyrotropin releasing hormone (TRH) during the estrous cycle

Day of estrous cycle	Pre-TRH prolactin (ng/ml)	Peak prolactin after TRH (ng/ml)	Time to peak (min)	Prolactin release after TRH[a] (ng ml^{-1}min)
0	8	73	13	1191 ± 408
2	3	61	12	812 ± 301
4	3	52	10	820 ± 179
7	9	77	12	1166 ± 169
15	5	74	11	1368 ± 225
18	6	81	17	1560 ± 247

[a]Integrated area of plot of prolactin concentrations for 30 min after TRH minus baselines before TRH. Values are means ± standard errors (n=6).

progesterone may be related to low serum prolactin is supported by the study of Reece and Bivens (1942) who reported that pituitary prolactin content was increased in ovariectomized rats following estrogen, progesterone or estrogen plus progesterone injections. In addition, there is another "tendency" for serum prolactin response curve areas and peak prolactin values after TRH to be larger at day 18 (proestrus) than at other days of the estrous cycle. This gives support to the concept that serum estrogen concentrations may affect serum prolactin concentrations since serum estrogen concentrations are increased at this time (Wettemann et al., 1972).

Although infusions of estradiol-17B in cows (Schams and Karg, 1972) and progesterone in bulls (Schams et al., 1974) increased serum prolactin concentrations, serum estrogen and progesterone concentrations

probably are of minor importance in the regulation of serum prolactin concentrations during the estrous cycle. This concept is supported by the study of Beck et al. (1976) who found that there were no differences in serum prolactin concentrations in ovariectomized heifers bearing estradiol-17B implants and/or progesterone pessaries. These authors suggested that neither estrogen nor progesterone alone or in combination influenced serum prolactin concentrations when present in blood at concentrations comparable with those found during the normal estrous cycle. In addition, Koprowski and Tucker (1973) found no differences in serum prolactin concentrations during the estrous cycle in lactating cows. In total, these data suggest that estrogen and progesterone are of minor importance in the regulation of circulating prolactin concentrations during the estrous cycle in heifers.

Serum GH concentrations before TRH administration averaged 6 ng/ml. Serum GH concentrations increased to maximum values of 18 to 24 ng/ml following injections of TRH (table 7). These maximal concentrations were attained 11 to 15 minutes after TRH was administered. Days of the estrous cycle did not affect ($P>.05$) the quantity of GH released, as measured by area under the response curve. These data are in agreement with Beck et al. (1976) who reported no differences in serum GH concentrations between ovariectomized control heifers and those heifers that had estradiol-17B implants and/or progesterone pessaries. These authors suggested, as with prolactin, that neither estrogen nor progesterone alone or in combination influenced serum GH when present in blood at concentrations comparable to those found during the normal estrous cycle.

Table 7.--Serum growth hormone (GH) response to thyrotropin releasing hormone (TRH) during the estrous cycle

Day of estrous cycle	Pre-TRH GH (ng/ml)	Peak GH after TRH (ng/ml)	Time to peak (min)	GH release after TRH[a] (ng ml^{-1} min)
0	5	24	12	283 ± 43
2	6	21	11	200 ± 96
4	6	18	11	187 ± 56
7	7	20	11	206 ± 46
15	7	20	14	193 ± 44
18	5	24	15	270 ± 40

[a]Integrated area of plot of growth hormone concentrations for 30 min after TRH, minus baseline before TRH. Values are means ± standard errors (n=6).

On the other hand, Koprowski and Tucker (1973) reported a small, but significant ($P<.05$), increase in serum GH concentrations around estrus as compared with the luteal phase of the estrous cycle in lactating cows. In addition, administration of diethylstilbesterol increased serum GH in steers (Trenkle, 1970) and injection of progesterone appeared to enhance the secretion of GH in ovariectomized ewes (Davis and Borger, 1974). In summary, although exogenous administration of estrogen or progesterone may increase serum GH concentrations, it is my opinion that estrogen and progesterone are of minor importance in the regulation of circulating GH concentrations during the normal estrous cycle in heifers.

Experiment 6. Effect of TRH on serum prolactin and GH during pregnancy

This experiment was designed to determine the effects of TRH on serum prolactin and GH concentrations at 3, 6 and 9 months of pregnancy at various seasons of the year.

Serum prolactin concentrations, prior to TRH administration, averaged 14 ng/ml. After TRH maximum serum prolactin concentrations ranged from 125 to 184 ng/ml and time required to attain these maximal values were 11 to 12 minutes (table 8). Stage of pregnancy did not affect ($P>.05$) the quantity of prolactin released, as measured by area under the response curve. However, pre-TRH concentrations, maximum concentrations after TRH and response curve areas for prolactin following TRH administration tended to be greater with advancing pregnancy. Month of year affected ($P<.01$) the quantity of prolactin released, as measured by area under the response curve (table 9). Prolactin response curve areas were greater ($P<.01$) in July when compared with response curve areas in November and April. However, response curve areas in November and April were not significantly different ($P>.05$) from each other.

The "tendency" for increased pre-TRH values, peak concentrations after TRH and response curve areas for prolactin with advancing pregnancy agrees with the data of Oxender et al. (1972) in heifers. Also, serum prolactin concentrations increased during late pregnancy in cows (Schams and Karg, 1970 and Johke et al., 1971) and ewes (McNeilly, 1971). In addition, Bates et al. (1935) and Reece and Turner (1937a) reported that pituitary prolactin content increased during late pregnancy in cattle.

Table 8.--Serum prolactin response to thyrotropin releasing hormone (TRH) during pregnancy

Month of pregnancy	Pre-TRH prolactin (ng/ml)	Peak prolactin after TRH (ng/ml)	Time to peak (min)	Prolactin release after TRH[a] (ng ml^{-1}min)
3	12	125	12	2205 ± 1309
6	14	148	12	2831 ± 1037
9	15	184	11	3155 ± 1210

[a]Integrated area of plot of prolactin concentrations for 30 min after TRH, minus baselines before TRH. Values are means ± standard errors (n=8 for heifers at 3 month stage of pregnancy; n=9 for heifers at 6 and 9 month stages of pregnancy).

Table 9.--Serum prolactin and growth hormone responses in pregnant heifers to thyrotropin releasing hormone (TRH) during seasons

Month of Year	Prolactin release after TRH[a] (ng ml^{-1}min)	Growth hormone release after TRH[a] (ng ml^{-1}min)
November	455 ± 115	121 ± 24
April	1193 ± 270	132 ± 27
July	7087 ± 1010	134 ± 21

[a]Integrated area of plot of hormone concentrations for 30 min after TRH, minus baselines before TRH. Values are means ± standard errors (n=9 for heifers during November and April; n=8 for heifers during July).

In contrast, Wettemannn and Hafs (1971) found that serum prolactin concentrations did not change significantly during the first 75 days of pregnancy in heifers. Arai and Lee (1967) reported a gradual decline in serum prolactin concentrations with advancing pregnancy in ewes. Davis et al. (1971) also found decreasing or low serum prolactin concentrations in ewes during advancing pregnancy, but serum prolactin concentrations began to increase gradually 3 to 5 weeks prior to parturition. In total, these studies indicate that serum prolactin concentrations remain relatively stable throughout most of pregnancy, but probably increase in late pregnancy. This increase may be important in the biochemical and anatomical changes that occur in the mammary gland prior to the initiation of lactation.

Month of year affected ($P<.01$) the quantity of prolactin released following TRH administration. This agrees with Koprowski and Tucker (1973) findings in lactating cows that serum prolactin concentrations were higher during April to September (74 ng/ml) than during October to March (35 ng/ml). Similarly, Schams and Reinhardt (1974) reported that serum prolactin concentrations were higher in summer, lower in winter in cattle. Furthermore, they found a significant correlation ($r=.9$; $P<.001$) between serum prolactin concentrations and the number of daylight hours. In addition, Tucker et al. (1974) reported that in bulls, blood serum collected in July contained more than twice as much prolactin as serum collected in January. These data demonstrate the existence of a seasonal effect on serum prolactin concentrations. Daily illumination and temperature are important factors in the seasonal cyclicity of serum prolactin concentrations. Increasing or decreasing the length of photoperiod increased or

decreased serum prolactin concentrations, respectively (Pelletier, 1973 and Bourne and Tucker, 1975). Similarly, increasing or decreasing temperature increased or decreased serum prolactin concentrations, respectively (Wettemann and Tucker, 1974). These effects are though to be mediated via the hypothalamus, but the exact mechanisms involved in this regulation are still unknown. Furthermore, the physiological significance, if any, of increased serum prolactin concentrations during summer, as compared with winter, remains a mystery.

Overall, baseline concentrations of GH in serum before TRH administration averaged 4 ng/ml. Maximum serum GH concentrations after TRH administration were 10 to 12 ng/ml and time required to attain these maximal values were 9 to 13 minutes (table 10). Neither stage of pregnancy (table 10) nor month of year (table 9) affected ($P>.05$) the quantity of GH released, as measured by area under the response curve. Lack of effects of stage of pregnancy on serum GH concentrations are in agreement with Oxender et al. (1972) who reported that serum GH concentrations did not change significantly during pregnancy in cows. Ingalls et al. (1973) observed no changes in serum GH concentrations between 26 and 9 days before parturition in heifers. However, serum GH increased as parturition neared and peaked at parturition. In contrast, Koprowski and Tucker (1973) found a small linear increase in serum GH concentrations with advancing pregnancy in lactating cows. In total, these data suggest that serum GH concentrations remain relatively stable throughout pregnancy, but probably increase just prior to parturition. This increase in serum GH concentrations just prior to parturition may be essential for the initiation of lactation since exogenous GH has been shown to be galactopoietic when given to lactating cows.

Table 10.--Serum growth hormone (GH) response to thyrotropin releasing hormone (TRH) during pregnancy

Month of pregnancy	Pre-TRH GH (ng/ml)	Peak GH after TRH (ng/ml)	Time to peak (min)	GH release after TRH[a] (ng ml^{-1} min)
3	4	11	13	132 ± 19
6	4	10	12	112 ± 24
9	4	12	9	142 ± 28

[a]Integrated area of plot of growth hormone concentrations for 30 min after TRH, minus baselines before TRH. Values are means ± standard errors (n=8 for heifers at 3 month stage of pregnancy; n=9 for heifers at 6 and 9 month stages of pregnancy).

In contrast to the prolactin data, serum GH concentrations following TRH administration were not affected (P>.05) by month of year. These results agree with Koprowski and Tucker's (1973) findings that serum GH concentrations were not affected by season of year in lactating cows. Tucker et al. (1974) observed that serum GH concentrations in bulls were not different in blood collected in July, as compared with blood collected in January. In addition, no significant differences were noted in serum GH concentrations in heifer calves exposed to 4.5, 21 or 32C for 9 days although GH concentrations tended to increase with increasing temperature (Tucker and Wettemann, 1976). These data suggest that seasonal effects on serum GH concentrations are minimal, and probably are of little importance in the regulation of serum GH concentrations.

Experiment 7. Effect of TRH on serum prolactin and GH during lactation

This experiment was designed to determine if serum prolactin and GH concentrations following TRH administration are affected at various stages of lactation within different months of the year and stages of pregnancy.

Each cow received TRH on two consecutive days at each stage of lactation to determine possible carryover effects on serum prolactin and/or GH concentrations. However, day of injection did not affect ($P>.05$) the quantity of prolactin released, as measured by area under the response curve (table 11). Therefore, data for days 1 and 2 were pooled for further analysis.

Prior to TRH administration, serum prolactin concentrations averaged 16 ng/ml. Maximum serum prolactin concentrations after administration of TRH ranged from 202 to 282 ng/ml at the five stages of lactation and time required to attain these maximal values were 12 to 16 minutes (table 12). Stage of lactation did not affect ($P>.05$) quantity of prolactin released. However, prolactin response curve areas following TRH administration tended to be greater at the 2-month stage of lactation (table 12). Analysis of variance showed that stage of pregnancy did not affect ($P>.05$) quantity of prolactin released, as measured by area under the response curve. Season of year affected ($P<.01$) the quantity of prolactin released, as measured by area under the response curve (table 13). Prolactin response curve areas were greater ($P<.01$) at March-May, June-August and September-November when compared with response curve areas at December-February.

Table 11.--Serum prolactin and growth hormone (GH) response to thyrotropin releasing hormone (TRH) on two consecutive days during lactation

Stage of lactation (months)	Day of injection	Pre-TRH prolactin (ng/ml)	Prolactin release after TRH[a] (ng ml^{-1} min)	Pre-TRH GH (ng/ml)	GH release after TRH[a] (ng ml^{-1} min)
2	1	24	5341 ± 870	6	259 ± 43
	2	20	5056 ± 734	6	269 ± 43
4	1	17	3694 ± 614	4	152 ± 42
	2	18	3348 ± 472	4	116 ± 23
6	1	12	3321 ± 582	5	144 ± 28
	2	14	4165 ± 709	5	143 ± 35
8	1	13	4132 ± 502	4	157 ± 50
	2	12	3448 ± 592	4	137 ± 33
10	1	16	4113 ± 633	4	138 ± 38
	2	17	5335 ± 686	4	134 ± 47

[a]Integrated area of plot of hormone concentrations for 30 min after TRH, minus baselines before TRH. Values are means ± standard errors (n=16).

Table 12.--Serum prolactin response to thyrotropin releasing hormone (TRH) during lactation

Stage of lactation (months)	Pre-TRH prolactin (ng/ml)	Peak prolactin after TRH (ng/ml)	Time to peak (min)	Prolactin release after TRH[a] (ng ml^{-1}min)
2	22	282	16	5199 ± 792
4	18	202	15	3521 ± 521
6	13	210	12	3743 ± 575
8	12	234	12	3790 ± 361
10	16	241	13	4724 ± 606

[a]Integrated area of plot of prolactin concentrations for 30 min after TRH, minus baselines before TRH. Values are means ± standard errors (n=16).

Table 13.--Serum prolactin and growth hormone (GH) responses to thyrotropin releasing hormone (TRH) during seasons

Seasons	Prolactin release after TRH[a] (ng ml^{-1}min)	Growth hormone release after TRH[a] (ng ml^{-1}min)
Mar-May	4274 ± 368	156 ± 29
June-Aug	5836 ± 525	139 ± 36
Sept-Nov	5009 ± 516	213 ± 31
Dec-Feb	1782 ± 244	131 ± 29

[a]Integrated area of plot of hormone concentrations for 30 min after TRH, minus baselines before TRH. Values are means ± standard errors.

Analysis of covariance was performed to determine what effects were attributable to either stage of lactation, stage of pregnancy or season (month) of year. Each of these factors was used in turn as a main effect with the other two factors and their squared terms serving as the covariates. Squared terms were used as covariates because the raw means indicated that the response curves over time were curvilinear. As with analysis of unadjusted data, analysis of covariance showed that neither stage of lactation nor stage of pregnancy affected ($P>.05$) quantity of prolactin released following TRH administration (table 14). However, analysis of covariance showed, like analysis of unadjusted data, that month of year affected ($P<.01$) quantity of prolactin released following TRH administration (table 14). Koprowski and Tucker (1973) reported that serum prolactin concentrations increased in response to stimuli associated with milking and this response was largest at 8 weeks of lactation, but then qradually decreased as lactation advanced until at 32 weeks prolactin was no longer released in response to milking associated stimuli. These results agree with the "tendency" I found for increased prolactin response to TRH during early lactation. In addition, Johke (1970) reported a rapid increase in serum prolactin concentrations associated with the milking stimulus during early lactation in cows and goats. In total, these data indicate that serum prolactin concentrations are increased during early lactation following either TRH or milking stimuli. I speculate that this increased pituitary responsiveness during early lactation is associated with lactational performance since milk yields are maximum during early lactation.

Table 14.--Serum prolactin (PRL) response to thyrotropin releasing hormone (TRH) during lactation, pregnancy and month of year[a]

Lactation		Pregnancy		Month of Year	
Month	PRL release after TRH[b] (ng ml^{-1} min)	Month	PRL release after TRH[b] (ng ml^{-1} min)	Month	PRL release after TRH[b] (ng ml^{-1} min)
2	4622	0	3771	Jan.	1559
		1	5275	Feb.	1883
4	4177	2	4623	Mar.	5031
		3	4287	Apr.	3602
6	4234	4	4822	May	4122
		5	4011	June	6558
8	3304	6	4065	July	5916
		7	1602	Sept.	7467
10	4193	8	3410	Oct.	4229
				Nov.	3184
				Dec.	2423

[a]Each variable used as main effect with other two variables serving as covariates. Analyses were performed as three separate analyses because of high correlation (r=.83) between stage of lactation and month of pregnancy.

[b]Integrated area of plot of prolactin concentrations for 30 min after TRH, minus baselines before TRH. Values are means.

The correlation coefficient between average daily milk yield for the 5 days preceding TRH administration at each stage of lactation was -.01 ($P>.05$). Schams and Karg (1970) also found no correlation between serum prolactin concentrations and stage of lactation or milk yield. In contrast, Koprowski and Tucker (1973) reported that milk yield and serum prolactin concentrations in blood collected immediately after and 1 hour after milking were significantly ($P<.01$) correlated (r=.36 and .18, respectively). In total, these data suggest that serum prolactin concentrations following TRH or milking stimuli are only very

slightly associated with lactational performance throughout the entire lactational period, but may be more associated during early lactation.

Seasonal and stage of pregnancy effects on serum prolactin concentrations confirm the results in experiment 6 and the discussion of these effects will not be repeated.

Serum GH concentrations, prior to TRH administration, averaged 5 ng/ml. Maximum serum GH concentrations were 13 to 24 ng/ml at the five stages of lactation (table 15). Time required to attain these maximal values were 9 to 13 minutes. Since day of injection did not affect ($P>.05$) quantity of GH released, as measured by area under the response curve (table 11), data for days 1 and 2 were pooled for further analysis. One-way analysis of variance showed that stage of lactation affected ($P<.05$) quantity of GH released. GH response areas were greater ($P<.05$) at the 2-month stage of lactation when compared with 4-, 6-, 8- and 10-month stages. Stage of pregnancy did not affect ($P>.05$) quantity of GH released; however, stage of lactation and month of pregnancy were highly correlated ($r=.83$). Therefore, a least squares analysis of covariance was used to analyze effects of stage of lactation, month of pregnancy and season of year using in turn one of these variables as a main effect and the other two as covariates (table 16) with analyses of all possible combinations. Adjusting for the respective two covariates in separate analyses, neither the main effects of stage of lactation nor month of pregnancy was significant ($P>.05$) when the other was used as a covariate. Therefore, as reported above, the data were analysed by one-way analysis of variance for unbalanced data.

Table 15.--Serum growth hormone (GH) response to thyrotropin releasing hormone (TRH) during lactation

Stage of lactation (months)	Pre-TRH GH (ng/ml)	Peak GH after TRH (ng/ml)	Time to peak (min)	GH release after TRH[a] (ng ml^{-1} min)
2	6	24	13	264 ± 40
4	4	13	13	134 ± 30
6	5	16	11	144 ± 28
8	4	15	12	147 ± 37
10	4	14	9	136 ± 34

[a]Integrated area of plot of growth hormone concentrations for 30 min after TRH, minus baseline before TRH. Values are means ± standard errors (n=16).

Table 16.--Serum GH response to thyrotropin releasing hormone (TRH) during lactation, pregnancy and month of year[a]

Lactation		Pregnancy		Month of Year	
Month	GH release after TRH[b] (ng ml^{-1} min^{-1})	Month	GH release after TRH[b] (ng ml^{-1} min^{-1})	Month	GH release after TRH[b] (ng ml^{-1} min^{-1})
2	260	0	175	Jan.	124
		1	125	Feb.	182
4	144	2	133	Mar.	143
		3	110	Apr.	224
6	164	4	173	May	163
		5	182	June	167
8	142	6	205	July	121
		7	226	Sept.	215
10	110	8	161	Oct.	314
				Nov.	143
				Dec.	151

[a]Each variable used as main effect with other two variables serving as covariates. Analyses were performed as three separate analyses because of high correlation (r=.83) between stage of lactation and month of pregnancy.

[b]Integrated area of plot of GH concentrations for 30 min after TRH, minus baselines before TRH. Values are means.

Season of year did not affect (P>.05), using either one-way analysis of variance or analysis of covariance, the quantity of GH released, as measured by area under the response curve.

Numerous researchers have demonstrated that administration of exogenous GH enhanced previously existing lactation in cows (Cotes et al., 1949, Donker and Peterson, 1951, Chung et al., 1953, Bullis et al., 1965 and Machlin, 1973). Since exogenous GH is galactopoietic in cows, the association of increased GH response curve areas at the 2-month stage of lactation and near maximum milking performance at this time suggests that circulating GH may be insufficient for maximum milk production during early lactation.

The lack of effect of season or month of pregnancy on serum GH concentrations confirm the results in experiment 6.

GENERAL DISCUSSION

Serum prolactin and GH concentrations generally increase rapidly following IV injection of TRH. Peak concentrations are usually attained within 6 to 12 minutes post-TRH. When only peak hormone concentrations are used to evaluate pituitary responsiveness to TRH, the results are based only on one value per sampling period and this value may be rather variable depending upon the variation from cow to cow as to their "actual" peak and whether or not the hormone has had time to be distributed uniformily throughout all compartments of the vascular system. Therefore, to minimize this problem, a third degree least squares polynomial hormone response curve was calculated to determine total hormone release. This was usually based on 11 samples collected over a 30-minute period after TRH administration minus the baseline average of 4 to 7 samples collected 15 to 30 minutes before TRH injections. I think that this gives a more reliable estimate of hormone release following TRH administration.

Season of the year has been reported to have a dynamic effect on serum prolactin, but not serum GH, concentrations in cattle (Koprowski and Tucker, 1973 and 1973a and Schams and Reinhardt, 1974). Highest serum prolactin concentrations are found in summer and lowest in winter. Illumination (Bourne and Tucker, 1975) and temperature

(Wettemann and Tucker, 1974) have been reported to be important in regulation of serum prolactin concentrations. Since increased temperatures and increased daylight occur concurrently during summer with the reverse true in winter, it is not possible to differentiate between the two effects except under controlled environmental conditions. Consequently, any effects thought to be associated with either temperature or daylight will be discussed as seasonal effects.

Similarly to many other researchers, I found that season of year was very important in regulation of serum prolactin concentrations in all physiological states that were studied. Therefore, based on the results of my early experiments and on the results of other workers, subsequent experiments were designed to take into account seasonal effects during pregnancy and lactation. Even during the same season, increased serum prolactin concentrations were noticed in prepubertal heifers on days when temperatures were higher than normal for that time of year. Results from the estrous cycle experiment, which was conducted from mid-November to mid-December, clearly demonstrate the suppression of serum prolactin concentrations when compared with values obtained in other experiments during the summer. Season of year affected ($P<.01$) prolactin response curve areas in the pregnant heifer and lactating cow experiments. These results clearly demonstrate that season of year is a very important determinant of serum prolactin concentrations.

The physiological significance of increased serum prolactin concentrations during summer is unknown. Several stimuli that are reported to be "stressful" to cattle result in increased serum prolactin concentrations. Whether increased ambient temperatures and/or increased

daylight during summer can be considered "stressful" must await further investigation. Another plausable explanation may be associated with water metabolism. Prolactin is known to be associated with water metabolism in certain lower species (Nicoll and Bern, 1972). Increased water consumption is known to occur in cattle during summer (Winchester and Morris, 1956). Therefore, increased serum prolactin concentrations may be associated with the increased water load that occurs. More research is needed in this area to explain the physiological significance of increased serum prolactin concentrations during summer.

Overall, neither serum prolactin nor GH concentrations were greatly affected by different physiological states. Pre-TRH serum prolactin concentrations averaged from 6 to 39 ng/ml in the seven experiments. However, if the experiment in prepubertal heifers, where increased serum prolactin concentrations were attributed to increased environmental temperature, and the estrous cycle experiment (conducted in Nov-Dec) were eliminated, pre-TRH serum prolactin concentrations were 13 to 22 ng/ml in virgin, pregnant or lactating cattle. These data suggest that different physiological states do not readily affect baseline prolactin concentrations. Similarly, pre-TRH GH concentrations (4 to 8 ng/ml) are not greatly different at the various physiological states that were studied. These data also suggest that baseline GH concentrations are not readily affected by different physiological states.

Routes of administration (IV, IM and SC) of TRH affected ($P<.01$) the quantities (response curve areas) of prolactin and GH released during a 30-minute post-TRH sampling period. In contrast, during a 2-hr post-TRH sampling period, neither prolactin nor GH

response curve areas were different ($P>.05$) following IV or SC administration of TRH. Following IV administration of TRH, serum prolactin and GH concentrations reach peak values more rapidly and decline more precipitously than following IM or SC administration of TRH. In total these results suggest that IM or SC injected TRH is retained within the depot sites and released from these stores relatively slowly over a period of time. Also, when time was allowed for serum prolactin and GH concentrations to return to baseline concentrations following TRH administration, hormone response curve areas were not different. Therefore, when a prolonged hormonal response is desirable, the SC or IM route of administration of TRH should be the method of choice.

Times required to reach maximum serum prolactin concentrations after IV administration of TRH in prepubertal heifers and postpubertal cattle were 7 to 10 minutes and 10 to 17 minutes, respectively. I speculate that these differences may be related to increased serum estrogen and/or progesterone concentrations in the postpubertal animals. These steroids may act to suppress TRH effects on the pituitary and thus delay prolactin release, but not affect the total amount of prolactin released.

Dose of TRH affected prolactin and GH response curve areas. The quantities (response curve areas) of prolactin and GH released increased linearly ($P<.01$) with increases in the log of the dose (5 to 100 µg) of TRH. Fell et al. (1973) and Noel et al. (1974) reported a dose-response increase in serum prolactin concentrations following TRH administration in ewes and humans. Maximal prolactin response curve areas were attained using 50 µg TRH. Convey (1973) reported a dose-response increase in serum GH concentrations following TRH

administration in lactating cows. Unlike prolactin, the largest GH response curve areas were attained using 100 μg TRH. However, it is possible that a larger dose of TRH would result in still greater GH response curve areas.

Peak GH concentrations and response curve areas following IV administration of TRH in pregnant heifers were slightly suppressed when compared with the responses in heifers during the estrous cycle and lactating cows. I speculate that these differences might possibly be related to estrogen and/or progesterone serum concentrations. Whether these differences are real and whether estrogen and/or progesterone are acting as moderators of TRH at the pituitary must await further research.

Johke (1970) and Koprowski and Tucker (1973) reported increased serum prolactin concentrations associated with milking stimuli. Correlation coefficients between serum prolactin concentrations prior to milking, immediately after milking and 1 hour after milking and milk yield were -.03 ($P>.05$), .36 ($P<.01$) and .18 ($P<.01$), respectively (Koprowski and Tucker, 1973). I found correlation coefficients ($P>.05$) of -.01 and .15 between milk yield throughout lactation (average of daily production for 5 days prior to TRH) and prolactin and GH response curve areas following TRH administration, respectively. Although both stimuli released prolactin, they probably act through different mechanisms and therefore it is not surprising to find a significant correlation associated with milking stimuli and not TRH. Johke (1970) and Koprowski and Tucker (1973) found a diminished prolactin response to milking stimuli in late lactation. In contrast, I found that serum prolactin concentrations increased in response to TRH at all stages of lactation that were tested. In particular, prolactin response curve

areas at the 10-month stage of lactation was 4,193 ng $ml^{-1}min$ (covariate adjusted mean). This value is as large as the average of serum prolactin response curve areas (4,084 ng $ml^{-1}min$) at 2-, 4-, 6- and 8-month stages of lactation. These results indicate that the pituitary is equally capable of releasing prolactin in late lactation as in other stages of lactation, although there was a "tendency" for greater prolactin release at the 2-month stage of lactation. The decreased prolactin release associated with milking stimuli in late lactation may possibly be related to increased hypothalamic PIF or biogenic amine concentrations. If this is so, even though the pituitary is capable of releasing prolactin, the pituitary may be under near constant inhibition and thus, the diminished prolactin release.

Nearly without exception, serum prolactin concentrations increased following TRH administration. In contrast, there were several animals that did not always have increased serum GH concentrations following TRH administration. This phenomenon has also been reported in humans (Saito et al., 1971). The GH response following TRH is not nearly as reliable as is the prolactin response. I have no explanation for this occurrence. The understanding of this phenomenon awaits further investigation.

In overview, season of the year is more important in determining serum prolactin, but not serum GH, concentrations than are any of the different physiological states that I studied. Furthermore, physiological states which I examined have relatively little effect on serum prolactin or GH concentrations.

SUMMARY AND CONCLUSIONS

Changes in serum prolactin and GH concentrations, following administration of TRH, were determined in prepubertal heifer calves, heifers during the estrous cycle and pregnancy, and in cows lactating 2 to 10 months. Parameters measured were: pre-TRH hormone concentrations, peak hormone concentrations after TRH, times required to achieve maximum hormone concentrations and integrated areas of plot of hormone concentrations for 30 minutes after TRH minus baselines before TRH.

Overall serum prolactin concentrations increased from pre-TRH concentrations of 16 ng/ml to 81 ng/ml following IV administration of 10 μg TRH in prepubertal heifers. Prolactin response curve areas ranged from 1,381 to 1,585 ng ml^{-1}min (P>.05). Repeatability coefficients for day to day baseline prolactin concentrations and prolactin response curve areas following TRH administration were .27 and .61, respectively. These results suggest that there are great differences from day to day in baseline prolactin concentrations and that there is less day to day variation in prolactin response curve areas following TRH administration. However, the correlation coefficient, within days, between baseline serum prolactin concentrations and maximum serum prolactin concentrations following TRH administration was .64 (P<.01). These results

suggest that on a given day there is a close relationship between baseline and peak prolactin concentrations.

Serum GH concentrations in prepubertal heifers prior to TRH averaged 8 ng/ml and increased to a maximum of 21 ng/ml after TRH. GH response curve areas ranged from 102 to 148 ng ml^{-1}min between days 1 and 8 ($P>.05$). Based on averaged GH response curve areas, there was little day to day variation in pituitary responsiveness to TRH. However, because of animal variation, averaged GH response curve areas are misleading. This is demonstrated by the low repeatability coefficient (.35) for GH response curve areas following TRH. Similarly, the day to day repeatability coefficient for baseline GH concentration was low (.37). Correlation coefficients between baseline GH concentrations and post-TRH maximum GH concentrations was .60 ($P<.01$). Similar to prolactin, post-TRH maximum GH concentrations on a given day are very dependent on baseline GH concentrations.

The quantity (response curve areas) of prolactin released increased linearly ($P<.01$) with increases in log of the dose (5 to 100 µg) of IV administered TRH. Peak serum prolactin concentrations increased from an averaged pre-TRH concentrations of 39 ng/ml to 143 to 277 ng/ml after TRH. Maximum quantities of prolactin released were achieved with 50 µg TRH.

Areas under the GH response curve increased linearly ($P<.01$) with increasing log of the TRH dose (5 to 100 µg). Pre-TRH GH concentrations averaged 5 ng/ml and increased to values of 11 to 46 ng/ml following TRH administration. The greatest dose of TRH tested (100 µg) resulted in the largest GH response curve areas, but may not have maximally stimulated the pituitary to release GH.

Following IV administration of TRH, serum prolactin concentrations increased to a greater peak (174 ng/ml) more rapidly and declined more precipitously when compared with either IM (105 ng/ml) or SC (107 ng/ml) administration of TRH with times required to attain those peak values being 9, 22 and 22 minutes, respectively. Prolactin response curve areas were 2,170, 1,322 and 798 ng ml^{-1}min following IV, IM and SC administration of 25 µg TRH, respectively, during a 30-minute post-TRH sampling period. In contrast, during a 2-hr post-TRH sampling period, prolactin response curve areas following IV or SC injections of TRH were 3,639 and 4,198 ng ml^{-1}min ($P>.05$), respectively. I concluded that route of administration affected peak serum prolactin concentrations and time required to attain these peaks, but not the total amount of prolactin released when time is allowed for serum prolactin concentrations to return to pre-TRH concentrations.

Maximum GH concentrations were 35, 25 and 14 ng/ml following IV, IM and SC administration of 25 µg TRH, respectively. Times required to attain these peak values were 12, 14 and 22 minutes following IV, IM and SC administration of 25 µg TRH, respectively. GH response curve areas were 326, 147 and 115 ng ml^{-1}min following IV, IM and SC administration of 25 µg TRH, respectively, during a 30-minute post-TRH sampling period. During a 2-hr post-TRH sampling period, GH response curve areas were 504 and 218 ng ml^{-1}min following IV and SC injections of 50 µg TRH, respectively. It was concluded that, similar to prolactin, route of administration affected maximum serum GH concentrations and times required to attain these peaks, but not the total amount of GH released when time is allowed for serum GH concentrations to return to pre-TRH concentrations. Therefore, when a prolonged

hormonal response is desirable, the SC or IM route of administration of TRH should be the method of choice.

Times required to reach maximum serum prolactin concentrations after IV administration of TRH in prepubertal heifers and postpubertal cattle were 7 to 10 minutes and 10 to 17 minutes, respectively. I speculate that these differences may be related to increased serum estrogen and/or progesterone concentrations in the postpubertal animals. They may act to suppress TRH effects on the pituitary and thus delay prolactin release, but not affect the total amount of prolactin released.

Serum prolactin and GH response curve areas did not differ ($P>.05$) following administration of TRH on days 0 (estrus), 2, 4, 7, 15 and 18 of the estrous cycle in heifers. However, there was a "tendency" for prolactin response curve areas, pre-TRH concentrations and maximum post-TRH concentrations at days 2 and 4 of the estrous cycle to be less than those at other stages of the estrous cycle. In contrast, there were no detectable effects of days of the estrous cycle on GH response curve areas. It was concluded that suppression of prolactin release from the pituitary at days 2 and 4 may be related to low serum estrogen and/or progesterone concentrations, but overall, neither serum estrogen nor progesterone plays a major role in regulation of serum prolactin or GH concentrations during the estrous cycle in heifers.

Neither prolactin nor GH response curve areas following TRH were affected ($P>.05$) by stage of pregnancy (3, 6 and 9 months). However, pre-TRH prolactin concentrations, maximum post-TRH prolactin concentrations and prolactin response curve areas after TRH "tended" to increase with advancing pregnancy. Whether this numerically greater

increase in prolactin is related to initiation of lactation awaits further study. Season of year did not affect (P>.05) the GH response curve areas following TRH administration. In contrast, prolactin response curve areas were affected (P<.01) by season of year being 6 to 15 X greater in summer than in fall and spring. I concluded that season of the year was probably of greater importance in determining serum prolactin concentrations than was pregnancy stage.

Neither stage of lactation (2, 4, 6, 8 and 10 months) nor month of pregnancy (concurrent with lactation) affected (P>.05) the quantity (response curve areas) of prolactin released following TRH administration. However, there was a "tendency" for greater prolactin release at the 2-month stage of lactation. In addition, month of year affected (P<.01) prolactin response curve areas following TRH administration. It was concluded from these data that the pituitary is more responsive to TRH during early lactation and that this increased responsiveness may be associated with increased milk yields at this early stage of lactation. Yet, when lactation was considered in its entirety, there was no correlation between prolactin and milk production (r=-.01).

Neither month of pregnancy nor month of year affected (P>.05) GH response curve areas following TRH administration. In contrast, stage of lactation affected (P<.05) the GH response curve areas following TRH administration. I concluded from these results that the pituitary is more responsive to TRH during early lactation and that circulating GH concentrations may not be sufficient for maximum milk production. However, overall correlation coefficients between milk production (average of 5 days preceding TRH injections) and hormone

response curve areas at the five stages of lactation were -.01 and .15 ($P>.05$) for prolactin and GH, respectively.

In overview, season of the year is more important in determining serum prolactin, but not serum GH, concentrations than are any of the different physiological states that I studied. Furthermore, physiological states which I examined have relatively little effect on serum prolactin or GH concentrations.

BIBLIOGRAPHY

BIBLIOGRAPHY

Adams, T. E., T. J. Rand and J. J. Reeves. 1973. Milk production in lactating rats treated with synthetic thyrotropin releasing hormone. J. Dairy Sci., 56:652.

Amenomori, Y., C. L. Chen and J. Meites. 1970. Serum prolactin levels in rats during different reproductive states. Endocrinology, 86:506.

Anderson, M. S., C. Y. Bowers, A. J. Kastin, D. S. Schalch, A. V. Schally, P. J. Snyder, R. D. Utiger, J. F. Wilber and A. J. Wise. 1971. Synthetic thyrotropin releasing hormone: a potent stimulator of thyrotropin secretion in man. New Eng. J. Med., 285:1279.

Arai, Y. and T. H. Lee. 1967. A double-antibody radioimmunoassay procedure for ovine pituitary prolactin. Endocrinology, 81:1041.

Armstrong, D. T. and W. Hansel. 1956. The effect of age and plane of nutrition on growth hormone and thyrotropic hormone content of pituitary glands of Holstein heifers. J. Anim. Sci., 15:640.

Bassett, J. M. and G. D. Thorburn. 1969. Foetal plasma corticosteroids and initiation of parturition in sheep. J. Endocrinol., 44:285.

Bates, R. W., O. Riddle and E. L. Lahr. 1935. Assay of three hormones present in anterior pituitaries of seven types of cattle classified for age, sex and stage of reproduction. Amer. J. Physiol., 113:259.

Beck, T. W., V. G. Smith, B. E. Seguin and E. M. Convey. 1976. Bovine serum LH, GH and prolactin following chronic implantation of ovarian steroids and subsequent ovariectomy. J. Anim. Sci., 42:461.

Blake, C. A. 1974. Stimulation of pituitary prolactin and TSH release in lactating and proestrus rats. Endocrinology, 94:503.

Blask, D. E. and R. J. Reiter. 1975. Pituitary and plasma LH and prolactin levels in female rats rendered blind and anosmic: influence of the pineal gland. Biol. Reprod., 12:329.

Boler, J. F., E. K. Folkers, C. Y. Bowers and A. V. Schally. 1969. The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline amide. Biochem. Biophys. Res. Comm., 37:705.

Bourne, R. A. and H. A. Tucker. 1975. Serum prolactin and LH responses to photoperiod in bull calves. Endocrinology, 97:473.

Bowers, C. Y., H. G. Friesen and K. Folkers. 1972. On the mechanism of TRH-induced prolactin release. Clin. Res., 20:71.

Bowers, C. Y. H. G. Friesen, P. Hwang, H. J. Guyda and K. Folkers. 1971. Prolactin and thyrotropin release in man by synthetic pyroglutamyl-histidyl-prolinamide. Biochem. Biophys. Res. Commun., 45:1033.

Brazeau, P., J. Rivier, W. Vale and R. Guillemin. 1973. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science, 179:77.

Brazeau, P., J. Rivier, W. Vale and R. Guillemin. 1974. Inhibition of growth hormone secretion in the rat by synthetic somatostatin. Endocrinology, 94:184.

Bryant, G. D., F. C. Greenwood, G. Kann, J. Martinet and R. Denamur. 1971. Plasma prolactin in the oestrus cycle of the ewe: effect of pituitary stalk section. J. Endocrinol., 51:405.

Bryant, G. D., F. C. Greenwood and J. L. Linzell. 1968. Plasma prolactin levels in the goat: physiological and experimental modification. J. Endocrinol., 40:iv-v.

Bullis, D. D., L. J. Bush and P. B. Barto. 1965. Effect of highly purified and commercial grade growth hormone preparations on milk production of dairy cows. J. Dairy Sci., 48:338.

Burd, L. I., J. A. Lemons, E. L. Makowski, G. Meschia and G. Niswender. 1976. Mammary blood flow and endocrine changes during parturition in the ewe. Endocrinology, 98:748.

Buttle, H. L. 1974. Seasonal variation of prolactin in plasma of male goats. J. Reprod. Fert., 37:95.

Buttle, H. L. and I. A. Forsyth. 1976. Placental lactogen in the cow. J. Endocrinol., 68:141.

Chambers, J. W. and G. M. Brown. 1976. Neurotransmitter regulation of growth hormone and ACTH in the Rhesus monkey: effects of biogenic amines. Endocrinology, 98:420.

Chen, C. L., Y. Amenomori, K. H. Lu, J. L. Voogt and J. Meites. 1970. Serum prolactin levels in rats with pituitary transplants or hypothalamic lesions. Neuroendocrinology, 6:220.

Chen, C. L. and J. Meites. 1970. Effects of estrogen and progesterone on serum and pituitary prolactin levels in ovariectomized rats. Endocrinology, 86:503.

Chung, A. C., J. C. Shaw and W. M. Gill. 1953. The effect of somatotropin upon milk production and various blood substances of lactating cows. J. Dairy Sci., 36:589.

Convey, E. M., J. W. Thomas, H. A. Tucker and J. L. Gill. 1973. Effect of thyrotropin releasing hormone on yield and composition of bovine milk. J. Dairy Sci., 56:484.

Convey, E. M., H. A. Tucker, V. G. Smith and J. Zolman. 1973. Bovine prolactin, growth hormone, thyroxine and corticoid response to thyrotropin-releasing hormone. Endocrinology, 92:471.

Cotes, P. H., J. A. Crichton, S. J. Folley and F. G. Young. 1949. Galactopoietic activity of purified anterior pituitary growth hormone. Nature, 164:992.

Cowie, A. T. 1969. General hormonal factors involved in lactogenesis. In Lactogenesis: The Initiation of Milk Secretion at Parturition. M. Reynolds and S. J. Folley, eds., p. 157. Univ. Pennsylvania press, Philadelphia.

Cryder, P. E., L. S. Jacobs and W. H. Daughaday. 1973. Regulation of growth hormone and prolactin secretion in patient with acromegaly and/or excessive prolactin secretion. Mt. Sinai J. Med., 40:402.

Davis, S. L. 1975. Somatostatin: its influence on plasma levels of growth hormone, prolactin and thyrotropin in sheep. J. Anim. Sci., 40:911.

Davis, S. L. and M. L. Borger. 1972. Prolactin secretion stimulated by TRH. J. Anim. Sci., 35:239.

Davis, S. L. and M. L. Borger. 1974. Dynamic changes in plasma prolactin, luteinizing hormone and growth hormone in ovariectomized ewes. J. Anim. Sci., 38:795.

Davis, S. L., L. E. Reichert, Jr., and G. D. Niswender. 1971. Serum levels of prolactin in sheep as measured by radioimmunoassay. Biol. Reprod., 4:145.

Debeljuk, L., A. Arimura, T. Redding and A. V. Schally. 1973. Effects of TRH and triiodothyronine on prolactin release in sheep. Proc. Soc. Exp. Biol. Med., 142:421.

Deis, R. P. and N. Alonso. 1973. Prolactin release induced by synthetic thyrotropin releasing factor in female rats. J. Endocrinol., 58:673.

Delouis, C. and R. Denamur. 1967. Experimental induction of a milky secretion during pregnancy in sheep. C. R. Acad. Sci., 2640:2493.

Deuben, R. R. and J. Meites. 1964. Stimulation of pituitary growth hormone release by a hypothalamic extract in vitro. Endocrinology, 74:408.

Dhariwal, A. P. S., L. Krulich and S. M. McCann. 1969. Purification of a growth hormone inhibiting factor (GIF) from sheep hypothalamus. Neuroendocrinology, 4:282.

Dickerman, E. 1971. Radioimmunoassay for rat growth hormone; further studies of the control of growth hormone secretion in the rat. Ph. D. Thesis, Michigan State University.

Dohler, K. D. and W. Wuttke. 1974. Serum LH, FSH, prolactin and progesterone from birth to puberty in female and male rats. Endocrinology, 94:1003.

Dohler, K. D. and W. Wuttke. 1975. Changes with age in levels of serum gonadotropins, prolactin and gonadal steroids in prepubertal male and female rats. Endocrinology, 97:898.

Donker, J. D. and W. E. Peterson. 1951. The milk secretion response of the bovine to injected growth hormone. J. Anim. Sci., 10:1074.

Everett, J. W. 1954. Luteotrophic function of autografts of the rat hypophysis. Endocrinology, 54:685.

Faglia, G., P. Beck-Peccoz, C. Ferrari, P. Travaglini, B. Ambrosi and A. Spada. 1973. Plasma growth hormone response to thyrotropin-releasing hormone in patients with active acromegaly. J. Clin. Endocrinol. Metab., 36:1259.

Fell, L. R., C. Beck, M. A. B. Blockey, J. M. Brown, K. J. Catt, I. A. Cumming and J. R. Goding. 1971. Prolactin in the dairy cow during suckling and machine milking. J. Reprod. Fert., 24:144.

Fell, L. R., J. K. Findlay, I. A. Cumming and J. R. Goding. 1973. Effect of synthetic TRF on prolactin release in sheep. Endocrinology, 93:487.

Folly, S. J. and F. G. Young. 1940. Further experiments on continued treatment of lactating cows with anterior pituitary extracts. J. Endocrinol., 2:226.

Forbes, J. M., P. M. Driver, A. A. El Shahat, T. G. Boaz and C. G. Scanes. 1975. The effect of daylength and level of feeding on serum prolactin in growing lambs. J. Endocrinol., 64:549.

Fredrikson, H. 1939. Endocrine factors involved in the development and function of the mammary glands of female rabbits. Acta Obstet. Gynecol. Scand., 19:1.

Friesen, H., P. Hwang, J. E. Tyson and A. Barbeau. 1972. Functional evaluation of prolactin secretion: a guide to therapy. J. Clin. Invest., 51:706.

Gautvik, K. M., B. D. Weintraub, C. T. Graeber, F. Maloof, J. E. Zuckerman and A. H. Tashjian, Jr. 1973. Serum prolactin and TSH: effects of nursing and pyroglu-his-proNH_2 administration in postpartum women. J. Clin. Endocrinol. Metab., 37:135.

Gotsulenko, B. R. 1968. Effect of gonadotropin, prolactin and sex hormones on the secretion of milk during their introduction into the mammary gland artery. Zootek. Fak. 23:6. Cited in Chem. Abst., 74:19 (1971).

Grosvenor, C. E. and F. Mena. 1973. Evidence for a time delay between prolactin release and the resulting rise in milk secretion rate in the rat. J. Endocrinol., 58:31.

Halasz, B., L. Pupp and S. Uhlarik. 1962. Hypophysiotrophic area in the hypothalamus. J. Endocrinol., 25:147.

Hart, I. C. 1973. Effect of 2-bromo-α-ergocryptine on milk yield and the level of prolactin and growth hormone in the blood of the goat at milking. J. Endocrinol., 57:179.

Hart, I. C. 1975. Seasonal factors affecting the release of prolactin in goats in response to milking. J. Endocrinol., 64:313.

Hart, I. C. and D. S. Flux. 1973. The release of growth hormone in response to milking in the goat during early and late lactation. J. Endocrinol., 57:177.

Hershman, J. M., A. Kojima and H. G. Friesen. 1973. Effect of thyrotropin-releasing hormone on human pituitary thyrotropin, prolactin, placental lactogen and chorionic thyrotropin. J. Clin. Endocrinol. Metab., 36:497.

Ieiri, T., Y. Akikusa and K. Yamamoto. 1971. Synthesis and release of prolactin and growth hormone *in vitro* during the estrus cycle of the rat. Endocrinology, 89:1533.

Ingalls, W. G., E. M. Convey and H. D. Hafs. 1973. Bovine serum LH, GH and prolactin during late pregnancy, parturition and early lactation. Proc. Soc. Exp. Biol. Med., 143:161.

Irie, M. and T. Tsushima. 1972. Increase of serum growth hormone concentration following thyrotropin-releasing hormone injection in patients with acromegaly or gigantism. J. Clin. Endocrinol. Metab., 35:97.

Jacobs, L. S., P. J. Snyder, J. F. Wilber, R. D. Utiger and W. H. Daughaday. 1971. Increased serum prolactin after administration of synthetic thyrotropin releasing hormone (TRH) in man. J. Clin. Endocrinol. Metab., 33:996.

Jobin, M., L. Ferland, J. Cote and F. Labrie. 1975. Effect of exposure to cold on hypothalamic TRH activity and plasma levels of TSH and prolactin in the rat. Neuroendocrinology, 18:204.

Johke, T. 1970. Factors affecting the plasma prolactin level in the cow and the goat as determined by radioimmunoassay. Endocrinol. Japon., 17:393.

Johke, T., H. Fuse and M. Oshima. 1971. Changes of plasma prolactin level during late pregnancy and early lactation in the goat and the cow. Jap. J. Zootech. Sci., 42:173.

Kamberi, I. A., R. S. Mical and J. C. Porter. 1971. Pituitary portal vessel infusion of hypothalamic extract and release of LH, FSH and prolactin. Endocrinology, 88:1294.

Kamberi, J. A., S. M. Renon and J. C. Porter. 1971. Effects of melatonin and serotonin on the release of FSH and prolactin. Endocrinology, 88:1288.

Kanematsu, S. and C. H. Sawyer. 1973. Elevation of plasma prolactin after hypophysial stalk section in the rat. Endocrinology, 93:238.

Kann, G. and R. Denamur. 1974. Possible role of prolactin during the oestrus cycle and gestation in the ewe. J. Reprod. Fert., 39:473.

Karg, H., D. Schams and V. Reinhardt. 1972. Effects of 2-Br-α-ergocryptine on plasma prolactin level and milk yield in cows. Experientia, 28:574.

Kelly, P. A., K. N. Bedirian, R. D. Baker and H. G. Friesen. 1973. Effect of synthetic TRH on serum prolactin, TSH and milk production in the cow. Endocrinology, 92:1289.

Kilpatrick, R., D. T. Armstrong and R. O. Greep. 1964. Maintenance of the corpus luteum by gonadotrophins in the hypophysectomized rabbit. Endocrinology, 74:453.

Kirk, R. E. 1968. Experimental Design: Procedures for the Behavioral Sciences. Brooks/cole Publishing Co., Belmont, CA.

Koprowski, J. A. and H. A. Tucker. 1971. Failure of oxytocin to initiate prolactin or lutenizing hormone release in lactating dairy cows. J.Dairy Sci., 54:1675.

Koprowski, J. A. and H. A. Tucker. 1973. Serum prolactin during various physiological states and its relationship to milk production in the bovine. Endocrinology, 92:1480.

Koprowski, J. A. and H. A. Tucker. 1973a. Bovine serum growth hormone, corticoids and insulin during lactation. Endocrinology, 93:645.

Krulich, L., A. P. S. Dhariwal and S. M. McCann. 1968. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary in vitro. Endocrinology, 83:783.

Lamming, G. E., S. Moseley and J. R. McNeilly. 1972. Prolactin release in the ewe at parturition and first suckling. J. Endocrinol., 55:XXVII.

L'Hermite, M., G. Copininschi, J. Goldstein, L. Vanhaelst, R. Leclerco, D. D. Bruno and C. Rolyn. 1972. Prolactin release after injection of thyrotropin-releasing hormone in man. Lancet, 1:763.

Li, C. H. and H. M. Evans. 1944. The isolation of pituitary growth hormone. Science, 99:183.

Lloyd, H. M., J. D. Meares and J. Jacobi. 1973. Early effects of stilbesterol on growth hormone and prolactin secretion and on pituitary mitotic activity in the male rat. J. Endocrinol., 58:227.

Lu, K. H. and J. Meites. 1972. Effects of L-dopa on serum prolactin and PIF in intact and hypophysectomized, pituitary-grafted rats. Endocrinology, 91:868.

Lu, K. H. and J. Meites. 1973. Effects of serotonin precursors and melatonin on serum prolactin release in rats. Endocrinology, 93:152.

Lu, K. H., C. J. Shaar, K. H. Kortright and J. Meites. 1972. Effects of synthetic TRH on in vitro and in vivo prolactin release in the rat. Endocrinology, 91:1540.

Lyons, W. R. 1942. The direct mammotrophic action of lactogenic hormone. Proc. Soc. Exp. Biol. Med., 51:308.

Machlin, L. J. 1973. Effect of growth hormone on milk production and feed utilization in dairy cows. J. Dairy Sci., 56:575.

Maeda, K., Y. Kato, S. Ohgo, K. Chihara, Y. Yoshimoto, N. Yamaguchi, S. Kuromaru and H. Imura. 1975. Growth hormone and prolactin release after injection of thyrotropin-releasing hormone in patients with depression. J. Clin. Endocrinol. Metab., 40:501.

Maeda, K., Y. Kato, N. Yamaguchi, K. Chihara, S. Ohgo, Y. Iwasaki, Y. Yoshimoto, K. Moridera, S. Kuromaru and H. Imura. 1976. Growth hormone release following thyrotropin-releasing hormone injection into patients with anorexia nervosa. Acta Endocrinol., 81:1.

McNeilly, J. R. 1971. A solid phase radioimmunoassay for ovine prolactin. J. Endocrinol., 49:141.

McNeilly, J. R., S. R. Moseley and G. E. Lamming. 1972. Observations on the pattern of prolactin release during suckling in the ewe. J. Reprod. Fert., 31:487.

Meites, J. and J. A. Clemens. 1972. Hypothalamic control of prolactin secretion. Vit. Horm., 30:165.

Meites, J., P. K. Talwalker and C. S. Nicoll. 1960. Initiation of lactation rats with hypothalamic or cerebral tissue. Proc. Soc. Exp. Biol. Med., 103:298.

Mims, R. B., C. L. Scott, O. Modebe and J. E. Bethune. 1975. Inhibition of L-dopa-induced growth hormone stimulation by pyridoxine and chlorpromazine. J. Clin. Endocrinol. Metab., 40:256.

Minaguchi, H., J. A. Clemens and J. Meites. 1968. Changes in pituitary prolactin levels in rats from weaning to adulthood. Endocrinology, 82:555.

Mishkinsky, J., K. Khazen and F. G. Sulman. 1968. Prolactin-releasing activity of the hypothalamus in post-partum rats. Endocrinology, 82:611.

Morag, M., J. Shani, F. G. Sulman and R. Yagil. 1971. The effect of perphenazine on mammary development and milk yield in the dairy ewe. J. Endocrinol., 49:351.

Moseley, S. R., J. R. McNeilly and G. E. Lamming. 1973. Prolactin release in the ewe induced by a synthetic tripeptide. J. Endocrinol., 59:XLV.

Mueller, G. P., H. T. Chen, J. A. Dibbet, H. J. Chen and J. Meites. 1974. Effects of warm and cold temperatures on releas of TSH, GH and prolactin in rats. Proc. Soc. Exp. Biol. Med., 147:698.

Mueller, G. P., H. J. Chen and J. Meites. 1973. In vivo stimulation of prolactin release in the rat by synthetic TRH. Proc. Soc. Exp. Biol. Med., 144:613.

Nair, R. M. G., J. F. Barrett, C. Y. Bowers and A. V. Schally. 1970. Structure of porcine thyrotropin releasing hormone. Biochemistry, 9:1103.

Nandi, S. and H. A. Bern. 1961. The hormones responsible for lactogenesis in the C3H/c Crgl mice. Gen. Comp. Endocrinol., 1:195.

Negro-Vilar, A., L. Krulich and S. M. McCann. 1973. Changes in serum prolactin and gonadotropins during sexual development of the male rat. Endocrinology, 93:660.

Nicoll, C. S. 1971. Aspects of the neural control of prolactin secretion. In Frontiers in Neuroendocrinolgy. L. Martini and W. F. Ganong, eds., p. 291. Oxford Univ. Press, New York.

Nicoll, C. S. and H. A. Bern. 1972. On the actions of prolactin among the vertebrates: is there a common denominator? In Lactogenic Hormones. G. E. W. Wolstenholme and J. Knight, eds., p. 299. Churchill Livingstone, London.

Nicoll, C. S., R. P. Fiorindo, C. T. McKennee and J. A. Parsons. 1970. Assay of hypothalamic factors which regulate prolactin secretion. In Hypophysiotropic Hormones of the Hypothalamus: Assay and Chemistry. J. Meites, ed., p. 115. Williams and Wilkins Co., Baltimore.

Nicoll, C. S. and J. Meites. 1962. Estrogen stimulation of prolactin production by rat adenohypophysis in vitro. Endocrinology, 70:272.

Niswender, G. D. 1974. Influence of 2-Br-α-ergocryptine on serum levels of prolactin and the estrous cycle in sheep. Endocrinology, 94:612.

Nititovitch-Winer, M. and J. W. Everett. 1958. Functional restitution of pituitary grafts re-transplanted from kidney to median eminence. Endocrinology, 63:916.

Noel, G. L., R. C. Dimond, L. Wartofsky, J. M. Earll and A. G. Frantz. 1974. Studies of prolactin and TSH secretion by continuous infusion of small amounts of thyrotropin-releasing hormone (TRH). J. Clin. Endocrinol. Metab., 39:6.

Oxender, W. D. H. D. Hafs and L. A. Edgerton. 1972. Serum growth hormone, LH and prolactin in the pregnant cow. J. Anim. Sci., 35:51.

Parsons, J. A. 1969. Calcium ion requirements for prolactin secretion by rat adenohypophyses in vitro. Amer. J. Physiol., 217:1599.

Pelletier, J. 1973. Evidence for photoperiodic control of prolactin release in rams. J. Reprod. Fert., 35:143.

Porteus, S. E. and P. V. Malven. 1974. Stimulation of prolactin release in vivo by thyrotropin-releasing hormone (TRH) through direct action on the ectopic rat pituitary. Endocrinology, 94:1699.

Purchas, R. W., K. L. Macmillan and H. D. Hafs. 1970. Pituitary and plasma growth hormone levels in bulls from birth to one year of age. J. Anim. Sci., 31:358.

Ratner, A. and J. Meites. 1964. Depletion of prolactin-inhibiting activity of rats hypothalamus by estradiol or suckling stimulus. Endocrinology, 75:377.

Raud, H. R., C. A. Kiddy and W. D. Odell. 1971. The effect of stress upon the determination of serum prolactin by radioimmunoassay. Proc. Soc. Exp. Biol. Med., 136:689.

Ravault, J. P. and M. Courot. 1975. Blood prolactin in the male lamb from birth to puberty. J. Reprod. Fert., 42:563.

Reece, R. P. and J. A. Bivens. 1942. Progesterone effects on pituitary lactogen content on mammary glands of ovariectomized rats. Proc. Soc. Exp. Biol. Med., 49:582.

Reece, R. P. and C. W. Turner. 1937. Experimentally altering prolactin content of the rat pituitary. Proc. Soc. Exp. Biol. Med., 36:283.

Reece, R. P. and C. W. Turner. 1937a. The lactogenic and thyrotropic content of the anterior lobe of the pituitary gland. Mo. Agri. Exp. Stat. Res. Bull., 266.

Reece, J. J., A. Arimura and A. V. Schally. 1970. Serum levels of prolactin and luteinizing hormone (LH) in the ewe at various stages of the estrous cycle. Proc. Soc. Exp. Biol. Med., 134:938.

Reinhardt, V. and D. Schams. 1975. Prolactin release in dairy heifers relative to duration of teat stimulation. Neuroendocrinology, 17:54.

Reiter, R. J. 1974. Pituitary and plasma prolactin levels in male rats as influenced by the pineal gland. Endocrinol. Res. Commun. 1:119.

Reiter, R. J. 1975. Changes in pituitary prolactin levels of female hamsters as a function of age, photoperiod and pinealectomy. Acta Endocrinol., 79:43.

Relkin, R. 1972. Effects of variations in environmental lighting on pituitary and plasma prolactin level in the rat. Neuroendocrinology, 9:278.

Saito, S., K. Abe, H. Yoshida, T. Kaneko, N. Nakamura, N. Shimizu and N. Yanaihara. 1971. Effects of synthetic thyrotropin-releasing hormone on plasma thyrotropin, growth hormone and insulin levels in man. Endocrinol. Japon., 18:101.

Sar, M. and J. Meites. 1967. Changes in pituitary release and hypothalamic PIF content during the estrous cycle of rats. Proc. Soc. Exp. Biol. Med., 125:1018.

Sar, M. and J. Meites. 1968. Effects of progesterone, testosterone and cortisol on hypothalamic prolactin inhibiting factor and pituitary prolactin content. Proc. Soc. Exp. Biol. Med., 127:426.

Schalch, D. S. and S. Reichlin. 1966. Plasma growth hormone concentration in the rat determined by radioimmunoassay: influence of sex, pregnancy, lactation, anesthesia, hypophysectomy and extracellular pituitary transplants. Endocrinology, 79:275.

Schally, A. V., A. J. Kastin, W. Locke and C. Y. Bowers. 1967. Hypothalamic releasing and inhibiting factors. In Hormones in Blood. C. H. Gray and A. L. Bacharach, eds., 2nd ed., Vol. 1, p. 492. Academic Press, New York.

Schally, A. V., T. W. Redding, C. Y. Bowers and J. F. Barrett. 1969. Isolation and properties of porcine thyrotropin releasing hormone. J. Biol. Chem., 244:4077.

Schally, A. V., S. L. Steelman and C. Y. Bowers. 1965. Effect of hypothalamic extracts on release of growth hormone in vitro. Proc. Soc. Exp. Biol. Med., 119:208.

Schams, D. 1972. Prolactin levels in bovine blood, influenced by milking manipulation, genital stimulation and oxytocin administration with specific consideration of the seasonal variations. Acta Endocrinol., 71:684.

Schams, D. 1972a. Prolactin releasing effects of TRH in the bovine and their depression by a prolactin inhibitor. Horm. Metab. Res., 4:405.

Schams, D., U. Andreae, V. Reinhardt and H. Karg. 1974. The influence of TRH on release of prolactin and lactation in the bovine. Acta Endocrinol. (Kbh) Suppl. 1, 184:111.

Schams, D. and H. Karg. 1970. Utersuchungen uber prolaktin im rinderblut mit einer radioimmunologischen bestimmungsmethode. Zbl. Vet. Med., A, 17:193.

Schams, D. and H. Karg. 1972. The immediate response of the plasma prolactin level to oestrogen infusions in dairy cows. Acta Endocrinol. (Kbh) 69:47.

Schams, D. and V. Reinhardt. 1974. Influence of the season on plasma prolactin level in cattle from birth to maturity. Horm. Res., 5:217.

Schams, D., V. Reinhardt and H. Karg. 1974. The immediate response of the plasma prolactin level to oestrogen and progesterone infusions in bulls. Acta Endocrinol., 76:242.

Shaar, C. J. and J. A. Clemens. 1972. Inhibition of lactation and prolactin secretion in rats by ergot alkaloids. Endocrinology, 90:285.

Shaar, C. J. and J. A. Clemens. 1974. The role of catecholamines in the release of anterior prolactin in vitro. Endocrinology, 95:1202.

Sinha, Y. N., P. W. Selby, U. J. Lewis and W. P. Vanderlaan. 1972. Studies of GH secretion in mice by homologous radioimmunoassay for mouse GH. Endocrinology, 91:784.

Sinha, Y. N. and H. A. Tucker. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci., 52:507.

Smith, V. G., T. W. Beck, E. M. Convey and H. A. Tucker. 1974. Bovine serum prolactin, growth hormone, cortisol and milk yield after ergocryptine. Neuroendocrinology, 15:172.

Spellacy, W. N., W. C. Buhi and R. P. Bendel. 1969. Human growth hormone levels during the menstrual cycle. Am. J. Obstet. Gynecol., 104:1038.

Stachura, M. E., A. P. S. Dhariwal and L. A. Frohman. 1972. Inhibitory effects of purified hypothalamic extract (HTE) on rat growth hormone (GH) - synthesis and release in vitro. Abstract, 4th Int. Congr. Ser., No. 256, p. 83.

Stricker, P. and F. Grueter. 1928. Action du lobe anterieur de l'hypophyse sur la montee laiteuse. Compt. Rend. Soc. de Biol., 99:1978.

Sulman, F. G. and J. Twersky. 1948. Lack of effect of prolactin on milk production of cows. Refuah. Vet., 5:33.

Swanson, L. V., H. D. Hafs and D. A. Morrow. 1972. Ovarian characteristics and serum LH, prolactin, progesterone and glucocorticoid from first estrus to breeding size on Holstein heifers. J. Anim. Sci., 34:284.

Takahara, J., A. Arimura and A. V. Schally. 1974. Stimulation of prolactin and growth hormone release by TRH infused into a hypophysial portal vessel. Proc. Soc. Exp. Biol. Med., 146:831.

Takahara, J., A. Arimura and A. V. Schally. 1974. Suppression of prolactin release by a purified porcine PIF preparation and catecholamines infused into a rat hypophysial portal vessel. Endocrinology, 95:462.

Talwalker, P. K., C. S. Nicoll and J. Meites. 1961. Induction of mammary secretion in pregnant rats and rabbits by hydrocortisone acetate. Endocrinology, 69:802.

Talwalker, P. K., A. Ratner and J. Meites. 1963. In vitro inhibition of pituitary prolactin synthesis and release by hypothalamic extract. Amer. J. Physiol., 205:213.

Tashjian, A. H., Jr., N. J. Barowsky and D. K. Jensen. 1971. Thyrotropin releasing hormone: direct evidence for stimulation of prolactin production by pituitary cells in culture. Biochem. Biophys. Res. Commun., 43:516.

Taylor, J. C. and M. Peaker. 1975. Effect of bromocryptine on milk secretion in the rabbit. J. Endocrinol., 67:313.

Thatcher, W. W. 1974. Effects of season, climate and temperature on reproduction and lactation. J. Dairy Sci., 57:360.

Tomogane, H., K. Ota and A. Yokoyama. 1975. Suppression of progesterone secretion in lactating rats by administration of ergocornine and the effects of prolactin replacement. J. Endocrinol., 65:155.

Torjesen, P. A., E. Haug and T. Sand. 1973. Effect of thyrotropin-releasing hormone on serum levels of pituitary hormones in men and women. Acta Endocrinol., 73:455.

Trenkle, A. 1970. Plasma levels of growth hormone, insulin and plasma protein-bound iodine in finishing cattle. J. Anim. Sci., 31:389.

Tucker, H. A. 1971. Hormonal response to milking. J. Anim. Sci., 32:137, Suppl. 1.

Tucker, H. A., J. A. Koprowski, J. H. Britt and W. D. Oxender. 1974. Serum prolactin and growth hormone in Holstein bulls. J. Dairy Sci., 57:1092.

Tucker, H. A., D. T. Vines, J. N. Stellflug and E. M. Convey. 1975. Milking, thyrotropin-releasing hormone and prostaglandin induced release of prolactin and growth hormone in cows. Proc. Soc. Exp. Biol. Med., 149:462.

Tucker, H. A. and R. P. Wettemann. 1976. Effects of ambient temperature and relative humidity on serum prolactin and growth hormone in heifers. Proc. Soc. Exp. Biol. Med., 151:623.

Tyson, J. E., H. G. Friesen and M. S. Anderson. 1972. Human lactational and ovarian responses to endogenous prolactin release. Science, 177:897.

Udeschini, G., D. Cocchi, A. E. Paneral, I. Gil-Ad, G. L. Rossi, P. G. Chiodini, A. Liuzzi and E. E. Muller. 1976. Stimulation of growth hormone release by thyrotropin releasing hormone in the hypophysectomized rat bearing an ectopic pituitary. Endocrinology, 98:807.

Voogt, J. L., C. L. Chen and J. Meites. 1970. Serum and pituitary prolactin levels before, during and after puberty in female rats. Amer. J. Physiol., 218:396.

Welsch, C. W., M. D. Squiers, E. E. Cassell, C. L. Chen and J. Meites. 1971. Median eminence lesions and serum prolactin: influence of ovariectomy and ergocornine. Amer. J. Physiol., 221:1714.

Wettemann, R. P. and H. D. Hafs. 1973. LH, prolactin, estradiol and progesterone in bovine blood serum during early pregnancy. J. Anim. Sci., 36:51.

Wettemann, R. P., H. D. Hafs, L. A. Edgerton and L. V. Swanson. 1972. Estradiol and progesterone in blood serum during the bovine estrous cycle. J. Anim. Sci., 34:1020.

Wettemann, R. P. and H. A. Tucker. 1974. Relationship of ambient temperature to serum prolactin in heifers. Proc. Soc. Exp. Biol. Med., 149:908.

White, W. F., M. T. Hedlund, G. F. Weber, R. H. Rippel, E. S. Johnson and J. F. Wilber. 1974. The pineal gland: a supplemental source of hypothalamic-releasing hormone. Endocrinology, 94:1422.

Wilson, C. A. 1974. Hypothalamic amines and the release of gonadotropins and other anterior pituitary hormones. In Advances in Drug Research. N. J. Harper and A. B. Simmonds, eds., Vol. 8, p. 119. Academic Press, New York.

Winchester, C. F. and M. J. Morris. 1956. Water intake rates of cattle. J. Anim. Sci., 15:722.

Wrenn, T. R. and J. F. Sykes. 1953. The response of hormonally induced bovine mammary tissue to anterior pituitary hormones. J. Dairy Sci., 36:1313.

Yoshinaga, K., R. A. Hawkins and J. F. Stocker. 1969. Estrogen secretion by the rat ovary in vivo during the estrous cycle and pregnancy. Endocrinology, 85:103.